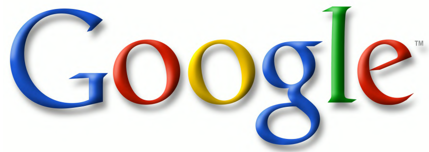

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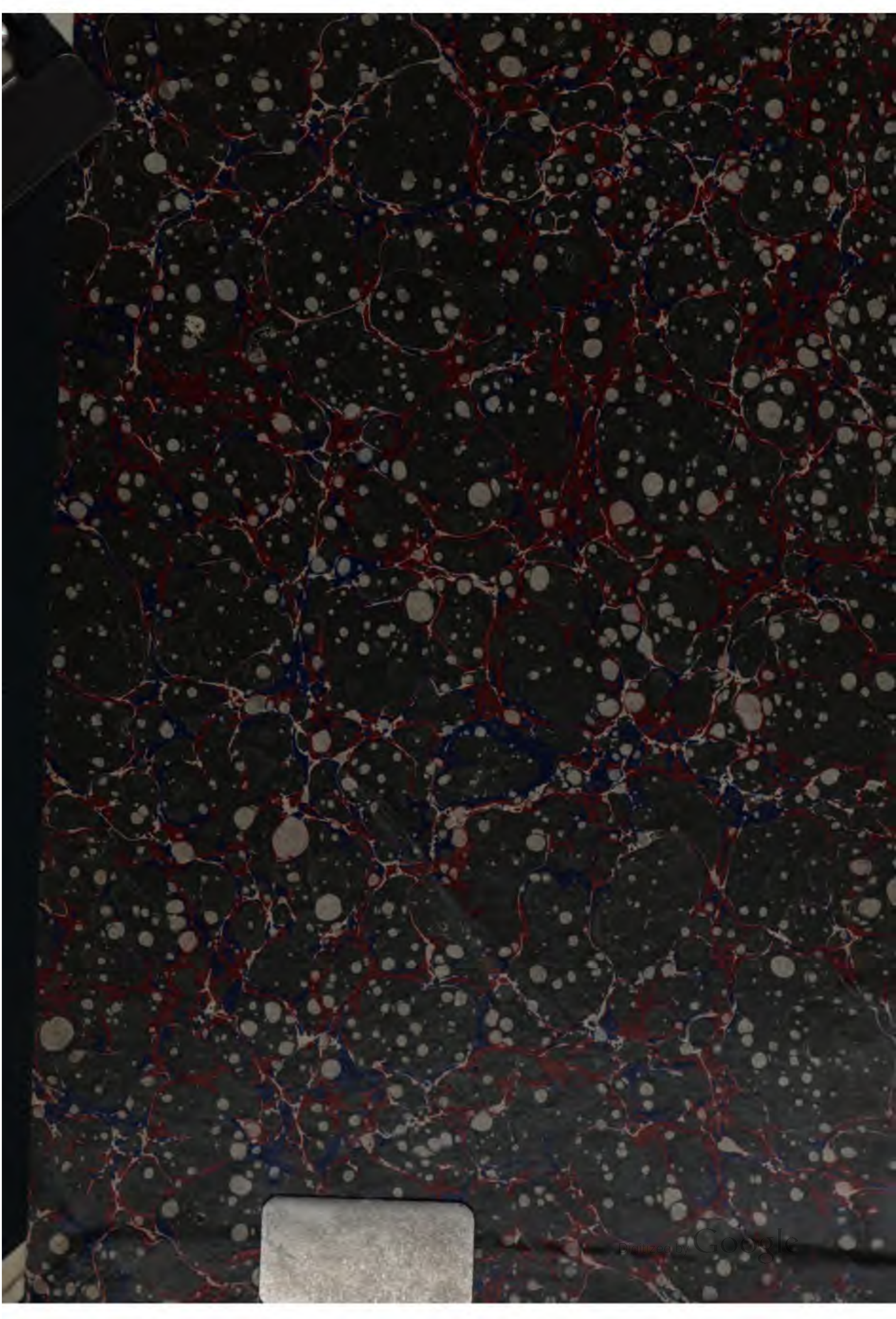
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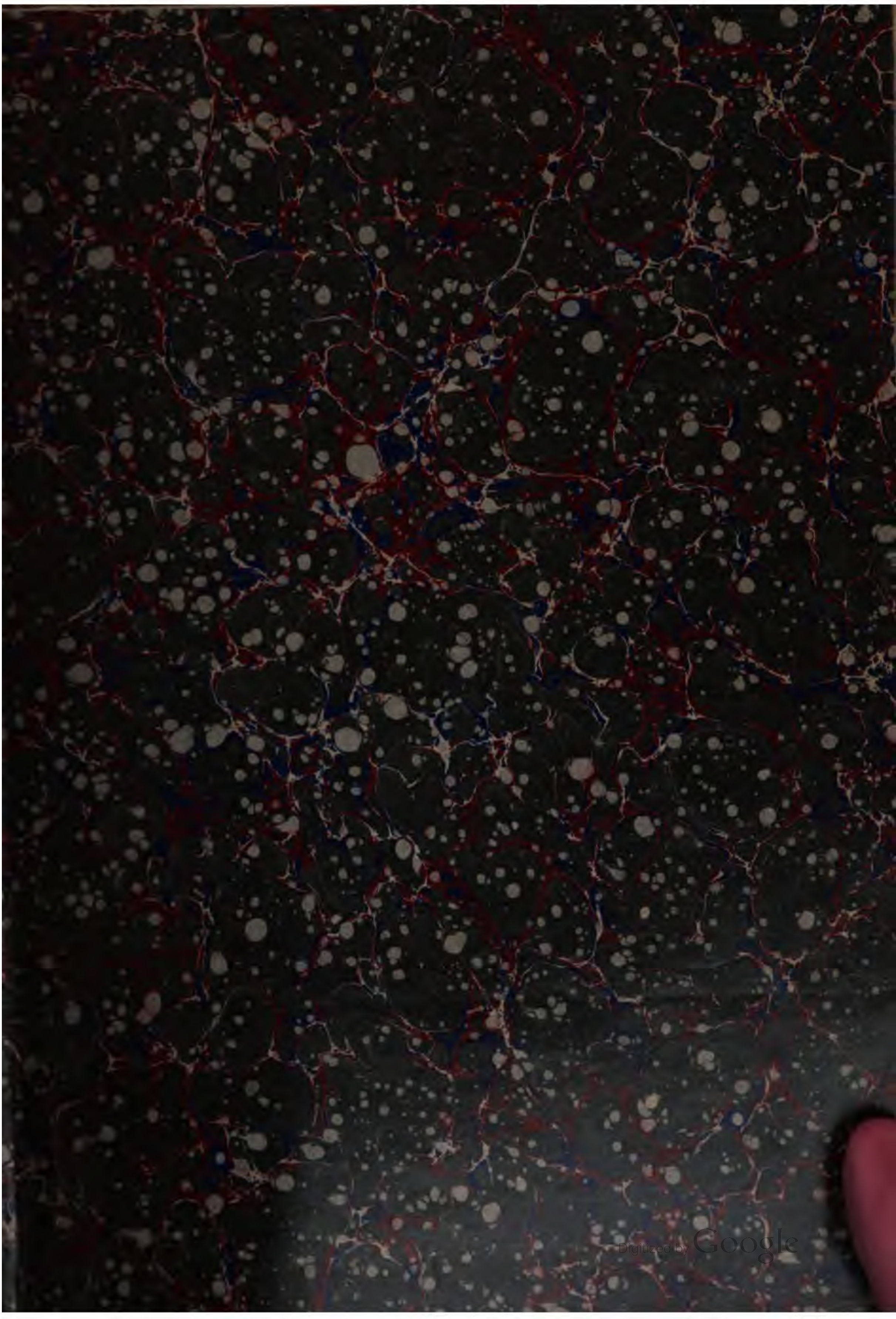
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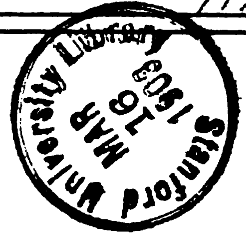


# THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

**The American Physiological Society**

BY

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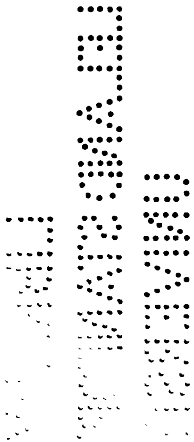

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# AMERICAN PHYSIOLOGICAL SOCIETY.

## SIXTH ANNUAL MEETING

IN CONNECTION WITH THE SIXTH CONGRESS OF AMERICAN PHYSICIANS AND SURGEONS.

WASHINGTON, D. C. MAY 12, 1903.

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# PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

## THE EFFECT OF ALCOHOL AND ALCOHOLIC FLUIDS UPON THE EXCRETION OF URIC ACID IN MAN.

By R. H. CHITTENDEN (for S. P. BEEBE).

Two kinds of experiments were tried: metabolism experiments, covering from one to three weeks; and hourly period experiments, in which the urine was collected each hour during the day. The subject of the metabolism experiments was a healthy young man of 65 kilos weight, not accustomed to the use of alcohol in any form. A fixed diet was eaten. Alcohol in four forms was used: absolute alcohol suitably diluted, whiskey, beer, and port wine. In most cases the quantity of the alcoholic fluid used in twenty-four hours contained between 75 and 80 c.c. of absolute alcohol. The most decided effect was obtained by the use of beer and port wine. There was but little difference between the effect of pure alcohol and whiskey. Typical results are as follows:

WHISKEY EXPERIMENT.

| | Nitrogen. | Ammonia N. | Uric acid. | Urea N.[1] |
|---|---|---|---|---|
| | grams. | gram. | gram. | per cent. |
| Fore period[2] . . | 15.61 | 0.741 | 0.554 | 89.6 |
| Alcohol period . . | 15.73 | 0.873 | 0.604 | 87.8 |
| After period . . . | 16.53 | 0.717 | 0.572 | 90.8 |

[1] Per cent of total nitrogen.
[2] These periods were of 6 days duration in each case, and the figures given are the daily averages for the periods.



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WINE EXPERIMENT.

| | Nitrogen. | Uric acid. |
|---|---|---|
| | grams. | gram. |
| Fore period . . . | 16.07 | 0.560 |
| Alcohol period . . | 16.84 | 0.773 |
| After period . . . | 16.64 | 0.580 |

Since the amount of alcohol in the different experiments is very nearly the same, the larger effect of beer and wine must be due to something other than the alcohol they contain.

In the hourly period experiments, the subjects fasted from 6 P. M. until 12 M. or 1 P. M. the following day, at which time a test meal was eaten; this served as a control day, while for the alcohol day the same program was followed, with the exception of adding varying quantities of alcohol to the test meal. In all but one individual the results showed a marked increase in the uric acid excretion. This increase began in most cases in the second hour after the meal, and reached the highest point at the fifth hour. The total quantity of uric acid excreted during the twenty-four hours of the alcohol day was greater than that of the control day, showing that the increase immediately following the alcohol meal was not due to a mere hastening of the normal output.

It may be noted that in one experiment where 50 c.c. absolute alcohol, suitably diluted with water, were taken without any food after the usual period of fasting, a very marked diuresis was produced, but a decrease in uric acid was noted. This indicates that the effect produced is due to a disturbance in the metabolism of the purin bases of the food.

---

## THE EFFECT OF SALINE INJECTIONS, DIURETICS, AND NEPHRITIC POISONS ON THE CHLORIDE-CONTENT OF THE URINE IN THE DOG.

BY TORALD SOLLMANN.

THE factors studied can be arranged in four classes:

*Class I:* Those which diminish the per cent of chlorine (to about 0.020 per cent), but which cause considerable diuresis and consequently an increase in the absolute quantity of chlorine: Intravenous

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injections of solutions of urea, glucose, alcohol, and sodium acetate, ferrocyanide, phosphate and sulphate.

*Class II:* diminishing both the per cent and the absolute amount of chlorine: water, salt starvation.

*Class III:* No effect upon the chlorine: nephritic poisons, caffein, phlorhizin, laking, degree of diuresis, and (within the limits of the experiments) the quantity or concentration of the injected fluid.

*Class IV:* Increasing the per cent of chlorine, if this has been previously low: solutions of sodium nitrate, sulfocyanide, iodide, and presumably bromide.

The study of these factors shows that the essential cause of the diminished chloride-content of the urine is not an increased diuresis, nor the presence of a foreign salt, nor the dilution of the serum; but the only essential fact is a lowered per cent of chlorine in the serum.

Analyses of the serum show, however, that the chlorine of the urine is not proportional to the total chlorine of the serum. To reconcile these facts it is necessary to assume that two forms of sodium chloride exist in the serum, and that the one form (perhaps in combination with proteid?) is not secreted by the living kidneys, whilst the other form (free NaCl) is readily excreted. This conclusion is confirmed by the perfusion of excised kidneys with diluted blood and with saline solutions; whereas, in the latter case, the urine contains the same per cent chlorine as the perfusing solution, it only contains a fraction of the chlorine of the serum if blood is perfused.

The effect of the nitrate, sulfocyanide, and iodide is most readily explained by assuming that these anions displace the chlorine ion from the unfiltrable compound.

---

## THE CAUSE OF THE GREATER DIURETIC ACTION OF HYPERISOTONIC SALT-SOLUTIONS.

By TORALD SOLLMANN.

It is found that the diuretic effect of equimolecular salt solutions is (with a few exceptions) proportional to their osmotic pressure. It is shown that this is not sufficiently explained by the greater hydræmia.

Experiments on excised kidneys show that hyperisotonic solutions

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increase, whilst hypoisotonic solutions decrease or arrest, the blood flow and urine filtration in excised and dead kidneys. The superior diuretic effect of hyperisotonic solutions is therefore, at least in large part, purely physical, and is explained by the dehydration and shrinkage of the renal tissues, and the more rapid circulation which this produced. The diuretic effect of saline injection is also partly explained by the dilution and lessened viscidity of the blood, and the resulting quickened renal circulation.

---

## ON THE PHARMACOLOGICAL ACTION OF OPTICAL ISOMERS.

ARTHUR R. CUSHNY.

It is well known that living protoplasm is capable of differentiating between optical isomers, such as the sugars, and tartaric acids, and Fischer has recently shown that the ferments are also possessed of this power. The mammalian tissues also oxidize one of the isomers more rapidly than the other. My experiments were performed with lævo-hyoscyamine and the racemic form, atropine. It was found that these acted equally strongly on the terminations of the motor nerves in striated muscle in the frog, on the frog's heart muscle and on the central nervous system in mammals, while atropine had a more stimulant action on the central nervous system of the frog. Hyoscyamine acted almost exactly twice as strongly as atropine on the nerve terminations in the salivary gland, heart, and pupil in mammals, from which it was inferred that the racemic atropine liberates the two optically active forms when dissolved, and that the dextro-hyoscyamine is practically devoid of action in these organs. This was confirmed by examination of some dextro-hyoscyamine obtained from its discoverer, Dr. Gadamer. It was found that lævo-hyoscyamine acts 12–16 times as strongly on the salivary secretion as dextro-hyoscyamine, and about 12–14 times as strongly on the terminations of the inhibitory cardiac fibres. The terminations of the nerves in the salivary glands, heart, and pupil can therefore differentiate between these optical isomers.

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## ON SECRETIN AND LYMPH-FLOW.[1]

By LAFAYETTE B. MENDEL (with HENRY C. THACHER).

Mr. Thacher has sought to ascertain whether the metabolic activity of the glands stimulated by secretin may give rise to an increased flow of lymph from the thoracic duct comparable with that induced

| Time. | Lymph-flow in 10 minutes. | Total solids in lymph. | Ash in lymph. |
|---|---|---|---|
| | c.c. | per cent. | per cent. |
| 11.08–11.38 | 1.5 | 5.46 | 0.87 |
| 11.47–12.17, injection of 50 c.c. of secretin solution. | | | |
| 11.47–11.57 | 2.4 | | |
| 11.57–12.17 | 4.6 | 5.53 | 0.85 |
| 12.19–12.29 | 3.5 | | |
| 12.29–12.39 | 2.2 | 5.58 | 0.89 |
| 12.39–12.59 | 2.1 | | |
| 1.03–1.33, second injection of 50 c.c. of secretin solution. | | | |
| 1.02–1.12 | 2.3 | | |
| 1.12–1.22 | 2.7 | | |
| 1.22–1.32 | 3.1 | 6.12 | 1.04 |
| 1.32–1.42 | 4.7 | | |
| 1.48–1.58 | 4.5 | | |
| 1.58–2.08 | 3.0 | 6.20 | 0.84 |
| 2.08–2.18 | 3.9 | | |
| After a third injection of secretin the animal was killed by blowing air into its veins. A post-mortem lymph-flow was noted, as follows: | | | |
| 3.00–3.20 | 2.9 | | |
| 3.20–3.45 | 1.6 | 6.44 | 0.91 |
| 3.45–4.10 | 0.1 | | |

by other lymphagogues and independent of any changes in arterial pressure.

Fifty cubic centimetres of a secretin solution (prepared as directed

[1] Read by title.

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by Bayliss and Starling) were injected into the facial vein of a dog of 16.5 kilos in thirty minutes. The arterial pressure remained unchanged at 76–78 mm. of mercury during the entire time, with the exception of a few seconds after the inadvertent rapid injection of two cubic centimetres of the solution. Similar injections were subsequently made with the same results. Extracts from the protocols follow:

The injection thus gave rise to an increased flow of lymph somewhat richer in solids, — a feature characteristic of the action of certain lymphagogues. The absence of the typical blood-pressure effects which accompany albumose injections would lead one to attribute the lymphagogic action to the secretin. Since this experiment was performed, however, Falloise has published a series of observations carried out with similar purpose. He concludes that purified and albumose-free secretin fails to accelerate lymph-flow, although it may still induce the typical stimulation of the pancreatic and hepatic cells. We have not followed this experimentally. An increased flow of lymph as observed above entirely independent of alterations in general blood-pressure is, however, of interest in itself.

---

## THE INFLUENCE OF CHINIC ACID ON THE ELIMINATION OF URIC ACID.

By W. A. TALTAVALL and WILLIAM J. GIES.

OUR work thus far has shown that the uric acid output in the urine of dogs is not materially affected by the administration of chinic acid. We observed only a slight lowering of the small amounts of uric acid present in the urine to begin with. This result was obtained when the animal was in approximate nitrogenous equilibrium on a mixed diet consisting of hashed meat, cracker meal, lard, bone ash, and water, and after daily doses, for ten days, of chinic acid in amounts varying from 1 to 20 grams. These results were obtained before the recent publication of the data of Hupper's experiments on himself. They agree with this observer's conclusions that the therapeutic deductions of Weiss, Blumenthal, and others, in this connection, are without foundation.

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## PEPTIC PROTEOLYSIS IN ACID SOLUTIONS OF EQUAL CONDUCTIVITY.

By WILLIAM J. GIES.

NUMEROUS digestive experiments with various equidissociated acids, and with fibrin as the indicator, have invariably given results lacking quantitative agreement. Undigested residue, neutralization precipitate, and uncoagulable products were determined gravimetrically. With all conditions exactly the same for each mixture in a series, except the *character* of the acid, the digestive products differed not only in the rate of their formation, but also in their amounts. The digestive results were particularly discordant in mixtures containing relatively small amounts of pepsin acting for comparatively short periods of time. That the anions greatly modified the action of the common cation seems certain, the $SO_4$ anion being especially antagonistic in its influence.

The temperature of the digestive mixtures in each experiment was kept steadily at 25° C. The acids used thus far were of the same conductivity as a 0.2 per cent solution of hydrochloric acid.

I am much indebted to Mr. C. W. Kanolt, of the Department of Physical Chemistry of Columbia University, not only for the acid solutions already used, but for others about to be employed in additional experiments.

---

## ON NUCLEIC ACID.

By P. A. LEVENE.

FURTHER investigation into the composition of nucleic acids of different origin resulted in the author improving the process of obtaining the pyrimidin bases so that no silver need be used in order to obtain thymin and cytosin. On decomposition of the acids of the spleen and of the pancreas there were found three bases, namely: thymin, cytosin, and uracil. The acid derived from yeast yielded on hydrolysis, only two of the bases: uracil and cytosin.

---

## ON THE TRUE ELEMENTARY COMPOSITION OF PURIFIED ADRENALIN AND THE RELATION OF THIS SUBSTANCE TO EPINEPHRIN. By J. J. ABEL.

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On a Difference in the Effect between the Simple Cutting of the Cervical Sympathetic Nerve and the Removal of the Superior Ganglion. By S. J. Meltzer and Clara Meltzer.

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This journal, 1903, ix, p. 245.

On the Excretion of Strontium. By L. B. Mendel (for H. C. Thacher).

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THE

# American Journal of Physiology.

VOL. IX. MARCH 2, 1903. NO. I.

## STUDIES ON THE INFLUENCE OF ARTIFICIAL RESPIRATION UPON STRYCHNINE SPASMS AND RESPIRATORY MOVEMENTS.

BY WILLIAM J. GIES AND S. J. MELTZER.

[*From the Laboratory of Physiological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

### I. HISTORICAL.

PREVIOUS to the discovery of the effect of artificial respiration upon strychnine convulsions, the observation was made by Meissner and Richter (1) that artificial respiration in curarized animals will prevent the outbreak of strychnine convulsions even after the paralyzing influence of curare has worn off. These authors did not ascribe this favorable result to the effect of artificial respiration, but assumed that, during the period of rest enforced by curare, the strychnine was partly eliminated from the body and partly neutralized within the body.

Leube (2), however, came to a different conclusion. Under the direction of I. Rosenthal, Leube studied the alleged immunity of the chicken to strychnine. He found that if artificial respiration be instituted during a strychnine tetanus, the tetanus will soon give way. If the dose of strychnine be too large, or the artificial respiration last only a short time, the convulsions may return.

Uspensky (3), also working under the direction of Rosenthal, a year later studied the influence of artificial respiration upon the spasms brought on by other poisons. He found that the convulsions which



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followed poisoning with brucin, thebain or caffein may be inhibited by artificial respiration, but that artificial respiration has no influence upon convulsions following poisoning with nicotin or picrotoxin. The poisons of the latter group, though capable of producing spasms, do not increase reflex irritability, while those poisons, the convulsions of which are affected by artificial respiration, have the common characteristic that they do increase reflex irritability. It appears evident, therefore, that artificial respiration inhibits only such spasms as are of reflex origin.

From the results of the preceding experiments, Rosenthal (4) concluded that artificial respiration exerts its influence upon the spasms by means of the increased oxygenation of the blood. He compared this influence with the effects which artificial respiration exerts upon the mechanism of respiration itself, in the production of apnœa. In both cases the oxygen reduces the irritability of the central organs; in respiration it is the natural irritability of the respiratory centre in the medulla oblongata, whereas in strychnine poisoning it is the exaggerated irritability of the spinal cord.[1]

Shortly after the experiments by Rosenthal and his pupils had been published, Schiff (5) obtained essentially the same results. Schiff observed, also, that after prolonged artificial respiration a few animals survived very large doses of strychnine.

During the succeeding interval a few publications have dealt with the facts and views presented by Rosenthal and his pupils. There is one assertion on record which is in contradiction to previous statements of fact. This was made by Rossbach and Jochelsohn (7) in a brief preliminary communication, which was never supplemented by a full publication of their experiments. They claim that artificial respiration has no soothing influence whatsoever upon strychnine spasms. These observers make additional statements in this connection which are not in conformity with the general experience, but which need not be discussed here. All other investigators confirm, unreservedly, the fact that artificial respiration exerts an in-

[1] According to this view the favorable results in the experiments of MEISSNER and RICHTER were due solely to the artificial respiration. We should like to call attention, however, to the experiments of CH. RICHET (6) in this connection. RICHET found that after poisoning with large doses of strychnine, the life of the animal is greatly prolonged, if, in addition to the artificial respiration, curare is also administered. RICHET makes no reference to the work of either MEISSNER and RICHTER, or to that of ROSENTHAL and his pupils.

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hibitory influence upon the strychnine convulsions. There is, however, a divergence of opinion regarding the nature of this inhibitory influence.

Ebner (8) and Buchheim (9) stated that they were able to induce the same soothing effect by simple movements of the body and extremities of the animal, and denied, therefore, that oxygenation has anything to do with the favorable action. They believe that the muscular movements are the favorable factors in the relaxation of the spasms. L. Pauschinger (10), however, working under Rosenthal's direction, could easily dismiss this contention by showing that the authors simply employed the now well-known Schultze's method of instituting artificial respiration without opening the trachea.

Brown-Séquard (11), after confirming the fact that the convulsions may be relieved by artificial respiration, denied that the favorable effect is due, as Rosenthal believed, to a greater charging of the blood with oxygen. He was of the opinion that artificial respiration causes a mechanical stimulation of the nerves of the lungs, thorax, and diaphragm, and thus affects an inhibition of the reflex centres.

The statements of Brown-Séquard were contradicted by Filehne (12). Later we shall have occasion to return to the works of both these observers.

Rosenthal's view was supported by Ananoff (13), who, in a brief communication, reported that animals breathing pure oxygen show a greater resistance to the effects of strychnine.

From 1878 to 1900 there is no publication to be found bearing on this subject. In the last-named year this question was studied by Osterwald (14) in the Pharmacological Institute of Göttingen. Osterwald, like Ananoff, put animals under glass bell jars through which a stream of oxygen was conducted. Experiments with mice did not yield striking results, but a few positive results with guinea-pigs led Osterwald to the unreserved support of the opinion that the favorable influence of artificial respiration is due to the greater introduction of oxygen into the blood.

Similar experiments were made last year by Von Czyhlarz (15) with guinea-pigs as well as with rabbits. His experimental results may be more appropriately discussed farther on.

The present status of this subject is, then, as follows: It is now a well-established fact that artificial respiration may prevent the outbreak of convulsions due to strychnine poisoning, or may inhibit

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them if already present, provided the dose of strychnine be not too large. The striking feature of its action is the perfect relaxation of the convulsed muscles, the absence of any muscular rigidity or any kind of tremor.[1] Artificial respiration here, apparently, inhibits the artificial increase of reflex-irritability. It is now the consensus of opinion that this inhibition is produced by increased introduction of oxygen into the blood, and that the mechanical effect of the expansion of the lungs, suggested by Brown-Séquard, has no share in the result.

This position, did not appear to us to be entirely satisfactory, for the following reasons: the soothing influence of artificial respiration upon the increased reflex-irritability due to strychnine is apparently identical with its soothing influence upon respiration itself, *i. e.*, with the production of apnœa.[2] We have already stated above that Rosenthal, who may be said to be the discoverer of these phenomena, looked upon both as processes of identical character — the inhibition of the normal reflex-irritability in one and inhibition of the increased reflex-irritability in the other. As regards the causation of apnœa it now seems to be a settled conviction that this state can be brought about by hyperoxygenation as well as by the mechanical distention of the lungs. Why, then, should it be different with the inhibitory effect of artificial respiration upon the strychnine spasms?

Furthermore, the evidence upon which the present prevailing view is based does not appear to us to be entirely conclusive. The chief points in the evidence are: 1. Filehne's work in disproving Brown-Séquard's claims of the disappearance of the effect of artificial respiration after section of the cord or the vagi; 2. Ananoff's,

[1] Some writers, when speaking of the favorable effect of artificial respiration, mean simply that it prolongs life. Life can be prolonged by artificial respiration, however, even if the administered strychnine dose is very large: but then the tonic and clonic convulsions continue even during the most energetic artificial respiration.

[2] The inhibitory effect of artificial respiration upon the complex mechanism of respiration may show itself in several ways: 1. The animal stops its normal abdominal and thoracic respiratory movements. 2. The concomitant respiratory movements of mouth and nose stop during artificial respiration. 3. All respiratory movements remain quiet for some time immediately after discontinuation of the artificial respiration. Usually only the last form of inhibition is termed apnœa. It is obvious, however, that the arrest of the respiratory movements during artificial respiration also belongs to the inhibition phenomena, and ought to be included in the term apnœa.

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Osterwald's, and Von Czyhlarz's experiments in producing the same favorable effect by simple normal inhalation of pure oxygen.

Considering the last line of evidence first, we have to exclude at the outset the testimony of Ananoff. In his short communication Ananoff speaks only of artificial respiration prolonging life, and does not mention the absence of spasms during the process which, as remarked above, is the essential point.

Osterwald's successful experiments were made on guinea-pigs (animals which are very resistant to strychnine), and were few in number. These strictures appear the more important when we read them in the light of the results reported by Von Czyhlarz. This last-named observer made nine experiments with guinea-pigs. In each experiment one animal inhaled pure oxygen and the other (control) inhaled air. In four of these experiments both animals had only marked hyperæsthesia. In one both animals had tetanus and survived. In the remaining four experiments the oxygen animals had marked hyperæsthesia, whereas the control animals had non-fatal convulsions. In the majority of the experiments, therefore, there were hardly any differences between the oxygen-breathing animals and the control animals, while the differences observed in the minority of the experiments were only of a minor character.

Von Czyhlarz's experiments on rabbits are still more instructive. Here he records eight experiments. In three experiments both animals had non-fatal tetani. In two both had fatal tetani. In one the oxygen animal had a fatal tetanus, and the control survived. In the remaining two experiments the oxygen animals died, while the controls survived their tetani. We fail to see in any of these experiments with rabbits even the shadow of proof that the inhalation of oxygen can suppress in these animals the increased reflex-irritability due to strychnine poisoning. Now, all the successful experiments ever made with artificial respiration were upon rabbits! If, however, it be admitted that the results of the above experiments with pure oxygen do prove that the oxygenation of the blood can neutralize to some degree the effect of strychnine, they surely do not prove that the mechanical distention of the lungs has no share in the effects produced by the classical method of artificial respiration.

There remains the work of Filehne, which was conducted in contravention of the claims put forward by Brown-Séquard. The latter observer, as stated above, was of the opinion that the arrest of respiratory movements of the animal (apnœa), as well as the arrest

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of the spasms in strychnine poisoning, both of which artificial respiration is capable of effecting, are not due to hyperoxygenation of the blood. The arrest in each case was attributed to the mechanical irritation of the branches of the vagus, the phrenic "or other diaphragmatic nerves," caused by the forced insufflation of air into the lungs. In support of his view Brown-Séquard states that transverse section of the spinal cord above the origin of the phrenic nerves or below their origin, or even section of the vagi, removes the arresting influence which artificial respiration exerts upon the respiratory movements.

In contravention of these statements Filehne reports that he tested these claims in a series of experiments and could not confirm them. An analysis of Filehne's experiments reveals the fact, however, that no experiment was made in which he studied the arrest of strychnine spasms by artificial respiration in animals whose spinal cord was cut. His attempts in this line were confined to the demonstration of the presence of apnœa after the severance of the cord. Even in these he succeeded in cutting the cervical cord in only one experiment. In a few other experiments he tried to crush the cord in young animals by forcibly constricting the cervical column with a string. The crudity of such a method hardly inspires confidence in the results attained by it.

Filehne further records a few experiments in which artificial respiration arrested strychnine spasms after cutting the vagi. These experiments, however, as was pointed out by Filehne himself, seem also to demonstrate that the cutting of the vagi visibly impairs the favorable effect of the artificial respiration.

We see, therefore, that the experiments to show the effect of inhalation of pure oxygen are still far from being decisively in favor of the exclusive oxygen theory. We find, further, that Filehne's work cannot be considered a sufficient refutation of Brown-Séquard's mechanical theory. Apparently, much more work must be done before the questions raised here can be satisfactorily answered.

## II. Our Own Experiments with Sections of Cord and Vagi.

From the above analysis it is evident that the claims of Brown-Séquard have not yet been properly tested, and that they deserve, therefore, to be investigated anew.

Brown-Séquard believed that the insufflation of air into the lungs

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irritates the endings of the vagus as well as of the phrenic and "other diaphragmatic nerves," whatever the latter may be. He might as well have said, also, that the sensory nerves of the thorax wall and the pleura might be stimulated by the rhythmical pressure of the artificial respiration. A more suggestive conjecture would be that the rhythmical pressure upon the contents below the diaphragm irritates the splanchnic nerves. We now know that stimulation of the central ends of the splanchnic nerves causes inhibition of inspiration (16). Experiments which exclude only one set of nerves, while the other paths of innervation remain intact, afford inconclusive evidence that the mechanical irritation of the nerves has no share in the inhibiting effect of artificial respiration. Our experiments were therefore directed in the first place toward the study of the action of artificial respiration on animals in which the vagi were cut, and, at the same time, the spinal cord severed at one place or another.

*General method.* — The experiments were made on rabbits, which were kept stretched on a holder, and were under ether anæsthesia during the operations. The artificial respiration was administered by bellows through a tracheal tube. The bellows were fastened to the table on which we operated, and were manipulated by hand. An average of thirty uniform strokes per minute was maintained, which caused a pressure rarely exceeding 36 mm. Hg. Each stroke with the bellows caused a distinct jar of the table upon which the animal was resting, a fact of importance in our experiments. We employed strychnine nitrate. An extensive experience has taught us that white rabbits are more sensitive to strychnine than colored ones. We have found that 0.45 mgm. of strychnine nitrate per kilo is a surely toxic dose for a white rabbit, and 0.5 mgm. for a gray one. Although this knowledge might have sufficed, we employed controls in almost every experiment. While our main object was the study of the influence of artificial respiration upon the spasms of strychnine, we also made note of the relation of artificial respiration to apnœa under these conditions.

Abbreviated protocols of our various experiments are given below:

*Experiment I.* — Gray and white male rabbit, 1920 grams. Tracheotomy.

5.30 P. M. Cord cut between third and fourth vertebra.

5.34. Strychnine injected, 0.6 mgm. per kilo.

5.35. Artificial respiration started, 30 to 35 mm. pressure.

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*Experiment I — (continued).*

5.37. Both vagi cut.

6.07. Artificial respiration discontinued. Animal was watched for ten minutes longer, and then was removed from board. During the forty-seven minutes after the injection of the fatal dose of strychnine, the animal did not show even any hyperæsthesia due to strychnine, although the table was jarred, the rabbit untied and its paws squeezed in testing for reflexes of the paralyzed hind limbs, and the animal *even removed from the table.*

With the dose administered in Experiment I a normal animal would have succumbed to a fatal tetanus in less than thirty minutes! The inhibitory effect of the artificial respiration was distinctly manifest, although the nervous paths of the vagi and the splanchnici were cut off. However, during the entire period of artificial respiration, there was in this experiment no full suppression of the animal's own breathing. Further, the concomitant respiratory movements of mouth and nose continued, and became very pronounced after the vagi were cut. There was no sign of apnœa after stopping the artificial respiration. In short, artificial respiration produced no apnœa. Possibly the artificial respiration with only 30 to 35 mm. pressure was not strong enough to cause apnœa in this large animal. But it remains a noteworthy fact that in an animal in which the paths through the vagi and splanchnici were blocked, a certain degree of artificial respiration was sufficient to influence the strychnine spasms, but not to cause apnœa!

Later the same animal was given another injection of strychnine — 0.5 mgm. per kilo. It had distinct convulsions after sixteen minutes. The noteworthy fact was observed that the convulsions did not appear simultaneously in the anterior and the posterior parts of the animal, but occurred in the part above the section of the cord usually before the part below. Subsequently, when the animal recovered from these convulsions, it was killed by asphyxia. It again had convulsions, which appeared in the hind part later than in the front, both sets of convulsions apparently continuing independently of one another.

*Experiment II.* — Gray rabbit, 1030 grams. Tracheotomy.

5.37 P. M. Cord cut opposite third dorsal vertebra.

5.42. Injected strychnine, 0.67 mgm. per kilo.

5.44. Artificial respiration started.

5.46. Artificial respiration slackened, signs of convulsive movements appeared. Artificial respiration immediately increased, perfect rest again.

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*Experiment II — (continued).*

6.00. Both vagi cut, no independent respirations, but concomitant breathing appears and remains throughout artificial respiration.

6.08. Artificial respiration stopped, soon vibration in upper part, and gasps.

6.09. Artificial respiration resumed, followed by rest again.

6.14. Artificial respiration discontinued, apnœa for a few seconds.

6.15. Convulsions in front parts, not in hind parts.

6.16. Convulsion in hind legs, none in front; soon, however, opisthotonus and death.

In this experiment, with a still larger dose of strychnine, the artificial respiration could not abolish the convulsions permanently, but while it was continued, arrested them for thirty-three minutes, the animal being perfectly relaxed, and even without hyperæsthesia during the entire period. In this smaller animal artificial respiration suppressed the independent respirations of the animal, and even caused a very brief period of apnœa, but it had no effect upon the concomitant respiratory movements of mouth and nose after the vagi were cut.

*Experiment III.* — White rabbit, 1400 grams. Tracheotomy.

5.03 P. M. Cervical cord cut opposite fifth vertebra, paralysis of hind and fore legs; no voluntary breathing. Artificial respiration begun.

5.11. Strychnine injected 0.6 mgm. per kilo.

5.31. While handled, slight and brief spasms (?).

5.33. Both vagi cut, gasping and other concomitant respiratory movements cannot be suppressed; pinching of any part brings out a tetanic convulsion confined to that part and lasting only as long as the part is handled; reflexes.

5.45. Artificial respiration discontinued, brief apnœa, then attempts to breathe; convulsions in upper part alone, later in lower part alone.

5.46. Rabbit dead.

In white rabbits 0.6 mgm. strychnine per kilo is a rapidly fatal dose. For thirty-five minutes, while the artificial respiration lasted, there were no real convulsions, but throughout the entire experiment there was a marked reflex hyperæsthesia, upon which the artificial respiration had apparently only a moderate inhibiting influence. After the vagi were cut the artificial respiration could not longer arrest the strong concomitant respiratory movements.

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*Experiment IV a.* — Gray rabbit, 1840 grams. Tracheotomy.

5.17 P. M. Cord cut opposite fifth cervical vertebra. Rabbit collapsed, no voluntary respiration, and only faint heart-beat. Artificial respiration, elevation of rear end of rabbit holder, and compression of abdomen.

5.46. Animal fully recovered.

6.01. Strychnine injected (0.06 mgm. per kilo strychnine sulphate + 0.06 per kilo strychnine nitrate).

6.33. Both vagi cut. Extremities squeezed or pulled, board hit, table thumped, but no convulsions, and even no hyperæsthesia. Independent voluntary respiration continually present.

6.41. Artificial respiration discontinued, no apnœa, breathes well.

6.46 to 6.49. Short tetanic convulsions either in front extremities alone, with legs upward, or in the four extremities with front legs downward.

6.50. Tetanus, opisthotonus, and death.

*Experiment IV b.* — Control, gray rabbit, 1760 grams.

4.44 P. M. Cord cut between third and fourth dorsal vertebræ.

6.03. Injected strychnine (0.06 mgm. per kilo strychnine nitrate + 0.06 per kilo strychnine sulphate).

6.30. On striking table tetanus in all parts at once, opisthotonus and death.

Although the animal in Experiment IV a received a fatal dose of strychnine and was subjected to all sorts of irritations, it manifested neither convulsions nor hyperæsthesia during the entire time it received artificial respiration. The control animal, Experiment IV b, on the other hand, had a fatal tetanus when the table was struck, twenty-seven minutes after receiving the strychnine. Six minutes after discontinuance of artificial respiration the strychnine poisoning became manifest also in Experiment IV a. The artificial respiration apparently only inhibited an increase of reflex-irritability but did not destroy the poison in the body; neither was the strychnine sufficiently eliminated from the body during the period of artificial respiration to prevent tetanus.

In this experiment artificial respiration did not suppress the voluntary breathing, nor did it produce any apnœa after its discontinuance.

*Experiment V a.* — Gray and white rabbit, 1240 grams. Tracheotomy. Artificial respiration for three minutes, voluntary and concomitant breathing suppressed. Artificial respiration stopped, apnœa only two seconds. Artificial respiration resumed, voluntary and concomitant breathing suppressed in one minute. Artificial respiration stopped after two minutes, apnœa eight seconds.

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*Experiment Va — (continued).*

5.45 P. M. Cervical cord cut between fifth and sixth vertebræ, animal in good condition. Artificial respiration resumed, voluntary and concomitant respiration suppressed only after seven minutes. Artificial respiration stopped, apnœa eight seconds. Artificial respiration immediately begun again, no voluntary and concomitant movements.

5.57. Strychnine nitrate injected, 0.65 mgm. per kilo.

6.07. Vagi cut; voluntary and labored concomitant breathing set in, each suppressed after seven minutes.

6.34. Artificial respiration stopped, apnœa eight seconds. Animal observed until 6.52. Although extensively handled during the fifty-five minutes since strychnine was injected, no sign of hyperæsthesia.

Later on asphyxia was caused by inhalation of hydrogen, and again by clamping of the trachea. Tetanic convulsions appeared only in the anterior part; the legs were directed towards the head.

*Experiment Vb.* — Control, gray and white rabbit, 1250 grams.

6.03 P. M. Injected strychnine nitrate 0.63 mgm. per kilo.

6.27. Animal stiff.

6.29. Tetanic convulsion.

6.33. When lifted there was a convulsion which the animal survived.

In this experiment the effect of artificial respiration upon strychnine spasms was very plain. They were entirely suppressed during the fifty-five minutes of observation, although the control animal began to show a distinct strychnine effect even twenty-four minutes after injection. The inhibitory effect upon the respiration was retarded by section of the cord as well as by section of the vagi, but finally a distinct apnœa was attained.

In the last three experiments the vagi and splanchnici, as well as the sensory fibres of the pleura and thoracic wall, at least most of them, were separated from the respiratory centre, etc. The roots of the brachial plexus were apparently divided in two parts, for when convulsive movements occurred in the upper part alone, the front legs were directed toward the head.

*Experiment VIa.* — Gray and white male rabbit, 1550 grams. Tracheotomy. Artificial respiration (25–30 mms. Hg) for three minutes. Voluntary and concomitant breathing soon suppressed. Artificial respiration stopped, apnœa three seconds.

4.43 P. M. Cord cut "between fourth and fifth cervical vertebræ," breathing stopped. Artificial respiration begun. Heart, lid reflex, etc., all right. Concomitant breathing soon suppressed. Artificial respiration stopped,

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*Experiment VI a — (continued).*

apnœa fifteen seconds, soon "head breathing." Artificial respiration resumed again, head breathing soon suppressed.

4.57. Injected strychnine nitrate 0.7 mgm. per kilo.

5.16. Both vagi tied off, concomitant breathing appeared, but disappeared again after four minutes.

5.32. Artificial respiration stopped, apnœa fifteen seconds, then "head dyspnœa"; no hyperæsthesia otherwise. Artificial respiration again.

5.40. Artificial respiration stopped, apnœa fifteen seconds, then gradual development of head dyspnœa and asphyxia.

5.43. Heart stopped. No convulsions.

*Experiment VI b.* — Control, gray and white rabbit, 1050 grams.

5.01 P. M. Injected strychnine nitrate 0.7 mgm. per kilo.

5.07. Convulsions, did not survive.

In Experiment VI a when artificial respiration was stopped there appeared now and then a very faint indication of thoracic movement. Possibly it was produced passively by the dyspnœic contraction of the cervical muscles. The autopsy showed that the cord was severed at the lower border of the fourth cervical vertebra, but the cut was diagonal and possibly a few fibres of the phrenic escaped. At all events this experiment is a strong demonstration of the efficiency of artificial respiration in suppressing strychnine spasms, and in producing apnœa, even after the vagi, splanchnici, brachial plexus, and almost all of the phrenic nerves are excluded. Although the control animal had a convulsion six minutes after injection (0.7 mgm. per kilo), the animal in Experiment VI a manifested no sign of strychnine spasms either during the forty-three minutes of artificial respiration or during final asphxyia. Furthermore, there was no concomitant breathing during the artificial respiration, and an apnœic pause was present after each interruption.

*Experiment VII a.* — Gray female rabbit, 1120 grams. Tracheotomy.

5.20 P. M. Cord cut near upper border of fifth cervical vertebra, animal breathes normally. Artificial respiration for a few minutes, voluntary respiration persistent. Artificial respiration stopped, no distinct apnœa. Artificial respiration begun again.

5.30. Both vagi cut, labored, concomitant breathing; voluntary and concomitant breathing subsiding slowly.

5.35. Injected strychnine nitrate, 0.7 mgm. per kilo.

6.05. Artificial respiration discontinued, no apnœa. Animal observed five hours longer. Had no sign of strychnine poisoning. When then

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*Experiment VII a* — (*continued*).

given a comparatively large dose of strychnine, it had a number of short convulsions in either of the two parts, independently of one another.

*Experiment VII b.* — Control, gray female rabbit, 1050 grams. For better comparison had ether anæsthesia for a few minutes.

5.40. Injected strychnine nitrate 0.7 mgm. per kilo.

5.59. Convulsions, succumbed in six minutes.

This experiment again is a classical demonstration of the inhibitory effect of artificial respiration upon the strychnine spasms even after section of cord and vagi. The effect upon respiration was less pronounced.

*Experiment VIII a.* — Gray female rabbit, 1750 grams. Tracheotomy.

3.59 P. M. Artificial respiration begun, only very slight concomitant respiratory movements.

4.01. Cord cut between second and third cervical vertebræ, concomitant breathing greatly increased, after a few minutes decreased again.

4.09. Artificial respiration discontinued, apnœa for a few seconds, then dyspnœa. Artificial respiration again.

4.15. Animal recovered from anæsthesia. Injected strychnine nitrate, 0.7 mgm. per kilo. Heart-beat, lid reflex, etc., normal until 4.30, when heart-beats became slower and labored concomitant respiratory movements reappeared.

4.35. The respiratory movements rapidly diminished; no lid reflex.

4.37. Heart-beats faint.

4.39. Death.

During the fifteen minutes after injection the animal was perfectly normal, but there was no sign of hyperæsthesia.

*Experiment VIII b.* — Control, gray rabbit, 1700 grams.

4.18 P. M. Etherized (for comparison) and kept under ether until 4.23.

4.19. Injected strychnine nitrate 0.7 mgm. per kilo.

4.33. Tetanic dance.

4.36. Convulsion terminating fatally at once. Although this animal was under the influence of ether while strychnine was injected, fourteen minutes after injection it manifested the unmistakable effects of this drug.

In Experiment VIII a the vagi, splanchnici, and all thoracic nerves, including the phrenici, were excluded. Although the animal died early, it lived long enough, and was normal long enough, to demonstrate that the strychnine had no effect so long as the artificial respiration was continued. There was once also a distinct apnœic pause.

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*Experiment IX a.* — Gray female rabbit, 1750 grams. Tracheotomy.

4.00 P. M. Artificial respiration begun.

4.02. Injected strychnine nitrate, 0.7 mgm. per kilo. The voluntary respirations were completely suppressed. Six minutes of artificial respiration, concomitant breathing not completely suppressed.

4.07. Cord cut at third cervical vertebra. Heart-beat, lid reflex, etc., normal, concomitant respiratory movements increased; remained unsuppressed throughout entire experiment.

4.12. Vagi cut. Animal normal throughout the remainder of the experiment. There was apparently a hyperæsthesia in the lower part, pressing or pulling legs was followed by contraction or tremor in legs, but these continued only as long as pull or pressure lasted. Blowing on animal, hitting table, no effect. Tremor in abdominal muscles; they even seem to contract synchronously with artificial respiration, simulating superficial independent voluntary breathing.

4.42. Artificial respiration stopped, all contraction and tremor disappear immediately (are due apparently to the local stimulus of the artificial respiration); head dyspnœa appears. Artificial respiration resumed again. Extremities and tail repeatedly pinched, pulled, etc., response with local, short reflexes, either during stimulation or immediately after. Pinching ear or other parts of head produce no reflex, but voluntary motion, moving away.

5.03. Artificial respiration discontinued; head dyspnœa, but no other movement of body.

5.04. Slight movement, and later, vibration only in front legs.

5.05. Sudden tetanus in all four extremities, followed by clonic convulsions.

5.06. Artificial respiration resumed. Lid reflex and heart-beat soon normal again, no more convulsions.

5.10. Artificial respiration discontinued again.

5.12. Sudden tetanus. Artificial respiration at once, and tetanus stopped suddenly. This procedure was repeated several times with same result, but sometimes tetanus stopped even while there was no artificial respiration.

*Experiment IX b.* — Control, gray female rabbit, 1600 grams.

4.22. Strychnine nitrate, 0.7 mgm. per kilo.

4.35. Tetanic convulsions.

4.45. Blown on, immediately violent convulsion, succumbs.

In Experiment IX a the section of the cord was above the phrenici, and the influence of the vagi and all other nerves concerned was positively excluded. The animal had a dose of strychnine which proved

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fatal to the control rabbit in less than half an hour. Although the animal in Experiment IX a was continually handled, and the reflexes, etc., tested, for an hour, while artificial respiration lasted, there was no reaction which could be ascribed to the effect of strychnine. Pounding the table or blowing on the animal had no effect at all. Pinching or pulling a leg brought out a local reflex which was apparently due only to the increased reflex-irritability caused by the section of the cord. Pressing one hind leg, for instance, would bring out a short flexion or extension of the opposite, or of a front leg. The artificial respiration caused short contractions of the abdominal muscles. But pinching any part of the head caused no reflex-response. The strychnine, however, was not destroyed within the body, nor sufficiently eliminated from it. Soon after stopping the artificial respiration there appeared convulsions and tetani, which by their entire character were apparently due essentially to the strychnine and not to asphyxia, or at least not to asphyxia alone. But these convulsions also could be stopped instantly by artificial respiration.

The influence of artificial respiration upon apnœa was not carefully noted in this experiment, but the concomitant respiratory movements continued during the hour while the artificial respiration lasted, although their intensity gradually decreased.

Our first series of experiments brought out one positive result. The claim of Brown-Séquard, that section of the cord or of the vagi abolished the arresting influence which artificial respiration exerts upon strychnine spasms, is entirely unfounded. Not only does section of the vagi alone, or of the cord alone, fail to impair this influence, but even cutting the vagi, combined with such section of the cord as excludes all influences of the splanchnic, diaphragmatic, and thoracic nerves, apparently does not interfere with the inhibitory influence of artificial respiration upon strychnine spasms. There were no convulsions in any of our experiments as long as sufficiently strong artificial respiration was administered. In many experiments no convulsions appeared even after the artificial respiration was stopped, although in all cases doses of strychnine were employed which by control experiments were proved to be surely toxic and mostly fatal. In some experiments artificial respirations arrested instantly the tetanic convulsions which were permitted to break out.

The doses of strychnine which we employed were, however, not

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much above the toxic or fatal minimum. Possibly section of the cord or vagi does interfere somewhat with the degree of favorable influence which artificial respiration might exert under such conditions. Filehne, who admits some impairment due to the section of the vagi, does not state the weight of his animals. Possibly, however, the doses which he employed were a trifle too large. Overdosage might also explain the claims put forward by Brown-Séquard. But the description of his experiments is too brief to permit any very definite interpretation. In fact it is not even evident that Brown-Séquard's conclusions regarding the relations of the sections of cord, or vagi, to the arresting influence of artificial respiration upon strychnine spasms were derived from actual experiments, and that they were not mere inferences from the experiments he made on the production of apnœa.

Regarding the latter, our own experiments have indeed demonstrated that section of the cord and the vagi impairs more or less the production of apnœa by artificial respiration. In some cases after section of the cord, and especially after additional section of the vagi, neither the voluntary respirations nor the concomitant respiratory movements could be suppressed. This was observed in some of the larger animals. Possibly the degree of ventilation employed in our experiments was not sufficient to accomplish this end in an animal with a comparatively large thorax. However, in all the experiments, section of the cord, or of the vagi, even during artificial respiration, immediately brought out again the voluntary breathing of the animal and especially the concomitant respiratory movements. It invariably took a much longer time to suppress the latter after section than before it.

Our experiments also showed that while artificial respiration completely suppresses the increased reflex-irritability due to strychnine-poison, it does not interfere, at least not strikingly, with the increased reflex-irritability induced by section of the cord. In all cases we were able, with little or no difficulty, to produce distinct reflexive movements by pinching a leg, touching an eye, etc., the posterior extremities responding more readily than the anterior ones. In one case, with section above the phrenici, each blow of the bellows brought out a contraction of the abdominal muscles simulating spontaneous breathing, which ceased on stopping the artificial respiration.

We noticed also, in the cases in which mild convulsions appeared after artificial respiration was stopped, that the parts lying above the

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line of section of the cord and those lying below it had their convulsions independently of one another. They were mostly insynchronous. In the experiments in which section of the cord was near the fifth cervical vertebra, the interesting observation was made that when the convulsions occurred in the anterior part, the anterior legs took part in it by moving toward the head, and that when the posterior part was convulsed the anterior legs moved toward the tail, pressing against the body. When, however, a violent tetanus broke out, the spasm convulsed all parts nearly simultaneously.

Thus it is evident that our experiments have established the fact contended for, but not proved by Filehne, namely, that section of the cord and vagi does not interfere with the inhibitory influence which artificial respiration exerts on strychnine spasms. But does this fact prove that the inhibitory influence of artificial respiration is due to the chemical influence of the oxygenation of the blood and to this alone? Does this fact indicate that the mechanical act of rhythmical insufflation has no share in the inhibitory influence?

The persistence of the favorable influence observed after section of cord and vagi could only then serve as an irrefutable proof if the claim for the mechanical share had been restricted to a hypothesis that the inhibition acts either through the agency of the respiratory centre or through the inhibitory mechanisms of the brain. If this is what Brown-Séquard meant, his theory is surely disproved by our experiments. The favorable influence of artificial respiration against the increased irritability of the spinal cord continues even after the cord has been severed from the controlling parts above it. But why restrict our hypothesis? We know that any reflex may be inhibited within the spinal cord by any mechanical stimulation of any part of the body. We have also seen in our experiments that, in an animal with a severed cord, artificial respiration caused rhythmical contraction of the abdominal muscles. This fact shows that the insufflations into the lungs, and the consequent abrupt increase of pressure upon the organs within the thoracic cavity, result in stimulating also the dorsal nerves imbedded in the abdominal section of the body. Furthermore we know that this insufflation causes an inhibition of centres lying within the medulla (respiratory, vaso-motor, cardio-inhibitory centres). Why then should it not be assumed that the rhythmical insufflations into the lungs stimulate all nerves within the thoracic and abdominal regions and thus inhibit increased reflex-irritability in all parts of the cord?

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The hypothesis formulated by Brown-Séquard is certainly untenable. That the arrest of the spasms can be due to the mechanical stimulation of the endings of the vagi, the phrenic and "other diaphragmatic nerves" alone, our experiments with section of the vagi and the cord have proven conclusively. But no cutting of the cord is capable of disproving the hypothesis that rhythmical insufflation is a mechanical stimulus for all the nerves within the trunk, by means of which an inhibition is caused in every section of the spinal cord above a cut as well as below it.

The question, therefore, is still open: Does the mechanical element involved in artificial respiration have a share in the arrest of the strychnine spasms, just as it is now generally assumed that it has a share in the production of apnœa?

## III. Artificial Respiration with Hydrogen.

For the solution of this question a method presents itself which at first sight appears to be quite simple. Previous investigators who desired to prove that it is the chemical factor which causes the arrest of the spasms have tried to introduce the oxygen without the complication of the mechanical element. Desiring to test the efficiency of the mechanical factor, we sought to determine the effect of artificial respiration with its chemical factor removed; *i.e.*, artificial respiration with an indifferent gas. It was partly by this method, indeed, that the value of the mechanical element in the production of apnœa was ascertained. We have, therefore, endeavored to study the effect of artificial respiration with pure hydrogen upon the strychnine spasms.

*General method.* — The method we employed was comparatively simple. Bellows were connected on one side with a gasometer containing pure hydrogen, and on the other side with the trachea of the animal. The tube connecting the bellows with the gasometer contained a valve which permitted the entrance of the gas into the bellows, but prevented it from going back to the gasometer. The tube connecting the bellows with the trachea contained a valve permitting the escape of the gas in the direction of the trachea, but preventing its return to the bellows. The expiratory air escaped through a lateral tube submerged under water (Müller's water valve), by which arrangement air was prevented from entering into the trachea through the expiratory aperture during a voluntary inspiration. All the con-

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nections were carefully made air tight. Each suction of the bellows brought hydrogen into it, and each compression drove the hydrogen into the lungs. The pressure was regulated by means of a stop-cock carried by the expiratory tube, and it was registered by a manometer connected by a T tube with the bellows-trachea tube.

We had, of course, no expectation of being able to continue the exclusive inhalation of hydrogen long enough to prevent the development of the strychnine poisoning, in the same manner as we succeeded in preventing it by the artificial respiration of air. We had observed that when once a tetanus broke out in our experiments it could be suppressed instantaneously by artificial respiration. In fact this instantaneous effect appeared to us to be in favor of the theory of a mechanical effect, since an effect due to a sufficient increase of oxygen in the blood could hardly develop so promptly after the first few strokes with the bellows. We therefore had reasonable expectations of witnessing the same instantaneous effect when pure hydrogen would be insufflated, or at least of observing it, long before the unavoidable asphyxia would finally compel the discontinuation of this gas. However, the first preliminary experiment, to determine the effect of insufflation of pure hydrogen upon the production of apnœa, brought us a surprise.

*Experiment X.* — White rabbit, 1700 grams. Tracheotomy, connected with bellows and gasometer, expiratory tube submerged. Insufflation of hydrogen for a brief period, apnœa for a few seconds. Repeated a few times with same result. Encouraged by the absence of asphyxia, the insufflation was continued consecutively for eighteen minutes, during which time there was no voluntary breathing, no concomitant respiratory movements, and no perceptible cyanosis of visible mucous membranes. After discontinuing the insufflation of hydrogen an apnœa of fifteen seconds appeared, but this was followed immediately by rapid superficial breathing and very rapid, faint heart-beats. Artificial respiration with air improved this condition, but the animal soon died through an accident.

Eighteen minutes' insufflation of pure hydrogen without asphyxia! That was surely an unexpected result. Before discussing it, however, we should quote a few of these hydrogen experiments in which also toxic doses of strychnine were injected.

*Experiment XI a.* — White rabbit, 1240 grams. Tracheotomy.

4.30 P. M. Injected strychnine nitrate, 0.6 mgm. per kilo.

4.33. Trachea connected with bellows, etc. Continued insufflation without incident till 4.58, when tetanic convulsions set in. Continued

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*Experiment XIa — (continued).*

insufflation until 5.01 without favorable effect. Insufflation stopped, animal thoroughly asphyxiated.

5.05. Artificial respiration with air.

5.07. Discontinued, no apnœa, immediately rapid breathing, a minute later convulsions, which continued for a few minutes. Animal killed.

*Experiment XI b.* — Control, gray and white rabbit.

5.17. Injected strychnine nitrate, 0.5 mgm. per kilo.

5.30. Convulsions broke out.

The animal in Experiment XI a was a white rabbit which, as mentioned above, was more susceptible to strychnine than the gray and white one. It received a larger dose than the gray control animal. Nevertheless, the convulsions did not break out until twenty-eight minutes after the injection, while the control had convulsions thirteen minutes after the injection. In this experiment the insufflation, however, could not put off asphyxia longer than twenty-five minutes, and with the onset of asphyxia the convulsions broke out.

*Experiment XII a.* — White rabbit, 2600 grams. Tracheotomy.

4.51 P.M. Injected strychnine nitrate, 0.53 mgm. per kilo.

4.55. Trachea connected with bellows, etc.

5.11. Some spasmodic twitching (beginning dyspnœa?). Increased the number and energy of the ventilations, animal quiet again.

5.15. Both vagi cut, " head dyspnœa " sets in.

5.25. Voluntary breathing of the animal appears and gradually increases.

5.28. Insufflation of hydrogen stopped, no apnœa, very labored dyspnœic breathing.

5.42. Trachea clamped, death. No strychnine effect at any time.

*Experiment XII b.* — Control, white rabbit, 1970 grams.

4.29. Injected strychnine nitrate, 0.45 mgm. per kilo.

5.01. Convulsions, died in two minutes.

In Experiment XII a, the animal received more strychnine than the control, which succumbed thirty-four minutes after injection, but had no convulsions for forty-seven minutes; *i.e.*, during the time it was under observation. The slight twitchings which appeared sixteen minutes after injection were promptly suppressed by the increase in ventilation with hydrogen. The inhibitory effect upon respiration, however, was greatly diminished by the section of the vagi. The

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concomitant breathing set in immediately, and the voluntary breathing appeared soon also, and apparently would have terminated in asphyxia, if the hydrogen insufflation had not been discontinued.

*Experiment XIII a.* — White rabbit, 1660 grams. Tracheotomy.

4.48 P. M. Injected strychnine nitrate, 0.54 mgm. per kilo.

4.50. Trachea connected with bellows, etc. At no time voluntary or concomitant breathing, no sign of hyperæsthesia.

5.21. Insufflation of hydrogen stopped, brief apnœa, then normal breathing. Observed till 5.42, no convulsions.

*Experiment XIII b.* — Control, white rabbit, 1420 grams.

5.27. Injected strychnine nitrate, 0.49 mgm. per kilo.

5.53. Had convulsions, and died in about two minutes.

The control had a fatal tetanus in twenty-six minutes, while animal XIII a, with a larger dose, showed no strychnine effect for the fifty-five minutes it was kept under observation. The insufflation lasted for thirty-one minutes and exerted a distinct inhibitory effect upon the respiration.

The results we obtained in these experiments were extraordinary indeed. Not only could the effects of fatal doses of strychnine be completely prevented by insufflation of pure hydrogen, but the animal could be kept by such an uninterrupted insufflation, as was seen in Experiment XIII a, for thirty-one minutes without manifesting any signs of asphyxia, dyspnœa, or cyanosis.

We all know very well that spontaneous inhalation of hydrogen alone results in asphyxia almost immediately. This is an old, well-established fact, and we have tested it ourselves by the following direct experiments. When the trachea of an animal was connected directly with the gasometer, without the intervention of the bellows, the animal thus surely inhaling, spontaneously, pure hydrogen, asphyxia set in after thirty to forty-five seconds, and rarely as late as after sixty seconds. Apparently, then, it was the action of the bellows which deferred asphyxia so long.

The first thought which occurs is that the bellows were, after all, not perfectly air tight. We have tested them by letting the animal spontaneously inhale the hydrogen from the gasometer through the expanded bellows without ventilating them. The asphyxia was then, indeed, deferred a little longer than when the inhalation occurred without the intervening bellows. However, the gain was at the utmost a minute or two, and therefore the amount of air which could have found access to the bellows must have been at most

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very small. But even granting that during the sudden and forcible expansion of the bellows more air was sucked into them than during the voluntary breathing, the amount of air which was able to penetrate the pores must under all circumstances necessarily have been very small in proportion to the quantity of hydrogen which, under constant pressure, had free access through the open lumen of a wide tube. It must also be remembered that the animal not only had no asphyxia under these conditions, but also that it was constantly in a state of apnœa, — a state which occurs only, it is assumed, when the animal receives more air than normally.

We may add, also, that, according to Osterwald (17), a diminution of oxygen favors the outbreak of strychnine spasms. In our experiments, with surely diminished oxygen there was no sign of convulsions even with fatal doses of strychnine.

These experiments brought us more than we looked for. It was now no longer a simple question whether the mechanical factor of artificial respiration has a share in the inhibition of strychnine spasms. The question which confronted us was whether one of the fundamental and apparently definitely settled principles in the theory of respiration did not require revision.

Searching through earlier literature on the subject of respiration we discovered that we had touched upon a long-forgotten chapter in the discussion whether the absence of oxygen or the presence of carbon dioxide is the cause of inspiration.

In 1862 L. Traube (18) made experiments with insufflation of hydrogen on dogs, in the same manner as we have made them on rabbits, and found, as we did, that artificial respiration with pure hydrogen may be carried on for a long period (forty-six minutes in one experiment), the animal remaining all the while in a state of apnœa. On the other hand the addition of carbon dioxide to the air rapidly caused dyspnœa. Traube, in consequence of these observations, gave up his original idea, that the absence of oxygen is the stimulus for inspiration, and accepted the view that the real cause of respiration is the presence of carbon dioxide. Heidenhain and Krause (19) soon contradicted Traube's statement, and explained his conclusion by assuming that his bellows were not air tight.

Traube (20) repeated his experiments, oiled his bellows, and took all precautions, as he states, to prevent the entrance of air, and insisted on the correctness of his former results, attributing the failure of Heidenhain and Krause to some fault in their technique.

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Traube was contradicted also by Thiry (21),[1] and finally by I. Rosenthal (23). Rosenthal did not repeat Traube's experiments, but connected the trachea of the animal with a gasometer of special construction containing pure hydrogen, and found that the animals became rapidly asphyxiated. By special calculations Rosenthal arrived at the conclusion that air which contains only 1 per cent of oxygen is sufficient for the maintenance of the animal, an amount which presumably found its way into the bellows in Traube's experiment. That was the last word, at least the last we found recorded in this discussion.

We may add that Traube's technique suffers from still another objection. In his experiments the opening for expiration had no valve. The animal, therefore, could obtain, during inspiration, sufficient air through this opening, even if it were made very small. As long as it was large enough for expiration it was also sufficient for inspiration. We have established this fact by experiment. The trachea was connected directly with the gasometer while the expiratory tube was submerged: asphyxia in forty-five seconds. The expiratory tube was left free in the air, and the stop-cock turned so as to make the lumen permissibly narrow: the animal went on breathing without noteworthy impediment for some time.

Rosenthal's paper appeared in 1864, and at that time there had not yet arisen the question whether the mechanical distention of the lungs can cause inhibition of inspiration. The only question in the minds of the earlier investigators was whether absence of oxygen or presence of carbon dioxide is the stimulus of respiration. And as the simple inhalation of hydrogen caused asphyxia, this appeared to prove that it is the absence of oxygen which causes respiration. Traube's experiments, therefore, seemed to have no further object. The value of the mechanical element which distinguishes artificial respiration from spontaneous breathing had not yet been recognized. We now know, from the studies of Hering and Breuer, Head, Gad, Meltzer, and others, that the mechanical effect of the distention of the lungs has a distinct inhibitory influence upon respiration.

It is now, furthermore, the general consensus of opinion that both

[1] That, at least, is what THIRY states in his paper in the Zeitschrift für rationelle Medizin (iii), xxi, p. 25. It is not stated on what grounds the opinion is based. MIESCHER-RÜSCH (22), however, quotes THIRY from a French paper as saying that artificial respiration with air and hydrogen causes apnœa. This paper was not accessible to us.

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the presence of carbon dioxide, as well as the absence of oxygen, act as stimuli to the respiratory mechanism. But it is surely not the actual immediate need of oxygen for metabolic purposes which in the latter case is the stimulus. The blood and lymph and tissues are provided with a surplus of oxygen for actual oxidative necessities. It is the first intimation of a deficit in this sinking fund which acts as a warning signal,—as a stimulus for increased provision of oxygen, increased inspiration. Is it, then, inadmissible to assume that this warning, this stimulus resulting from diminution in the body's income of oxygen, could be overcome for some time by the inhibitory influence of the rhythmical mechanical effect of distention of the lungs, if sufficient provision were made for the full escape of the carbon dioxide? Our experiments do not, of course, warrant such a positive conclusion. The bellows permitted the entrance of air to some degree, but the amount of air which entered was surely comparatively small. If, therefore, our experiments, as well as those of Traube, do not yet permit positive conclusions in this regard, they are at least suggestive enough to urge the necessity of a reinvestigation of this particular question with more favorable methods. In this relation the necessity of avoiding suction apparatus in the execution of artificial respiration with indifferent gases seems important.

In this connection, also, we wish to call attention to the statement of Head (24) that he caused apnœa by insufflation of hydrogen. His conclusion was that the apnœa was due to mechanical effects. He used bellows, and does not mention any precaution taken to guard against the entrance of air into the bellows. Could not the contention be made against his conclusions also, as it was raised against Traube's, that it was the air which entered through the pores of the leather into the bellows that brought about the observed result?

Regarding the arrest of the strychnine spasm, which we observed, with hydrogen insufflations, it appears very probable that it is due largely to the mechanical effect of the insufflation, and that it is not essentially a result of the admixture of small amounts of air. Here also additional experiments, and by other methods, will have to be made before the question can be definitely settled.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 140.

# ON THE NEGATIVE AND POSITIVE PHOTOTROPISM OF THE EARTHWORM ALLOLOBOPHORA FŒTIDA (SAV.) AS DETERMINED BY LIGHT OF DIFFERENT INTENSITIES.

BY GEORGE P. ADAMS.

THAT earthworms are negatively phototropic, that is, creep away from a source of light, has been frequently demonstrated, and the extent to which this is true has already been roughly estimated for Allolobophora fœtida. Parker and Arkin (:01, p. 154) found that when this worm was illuminated on the whole of one side by a light of 3.3 to 4.0 candle-metres intensity, 30.2 per cent of the head movements were away from the light. Of these, however, 4.2 per cent were due to other deflecting causes than light, and hence 26 per cent may be taken as a measure of the deflecting effect of the light. Miss Smith (:02, p. 469) carried out similar experiments, but by slightly different methods, and found that 61 per cent of the reactions were in a generally negative direction. If Miss Smith's results are interpreted in the way in which Parker and Arkin's were, it would appear that 61 per cent less 22 per cent, or 39 per cent, is the proportion of reactions to be attributed to the deflecting effect of the light. Thus, there is a disagreement in the two sets of records amounting to the difference between 39 per cent and 26 per cent. This disagreement may be due to differences in the intensities of the light used in the two sets of experiments. Miss Smith carried on her experiments in diffuse daylight, but as she gives no data from which the intensity can be calculated, this assumption may or may not be correct.

The present investigation was made under the direction of Dr. G. H. Parker, to ascertain the relation between the intensity of the light to which the earthworm Allolobophora fœtida (Sav.) was exposed, and its negative phototropic reactions; and to find out under what conditions, if any, the reactions would become positive. Inversions in the sense of the phototropism have already been recorded for a



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number of animals. Thus, Wilson ('91, p. 414) has shown that Hydra is negative to bright light, and positive to dim light; and the same has been demonstrated for Polygordius larvæ by Loeb ('93, p. 89), for Limax by Frandsen (:01, p. 206), and for the females of the Copepod Labidocera æstiva by Parker (:02, p. 114). In these instances negative phototropism is associated with an increased intensity of light, and this seems to be a general rule, though there is at least one exception to it, namely, in Orchestia agilis. This crustacean, as reported by Holmes (:01, p. 216), becomes positive in bright light, but is subsequently negative when transferred to diffuse daylight.

In testing Allolobophora fœtida, twelve different intensities of light were used, ranging from 192 candle-metres to 0.001 candle-metre. The first set of experiments was made with one piece of apparatus, using eleven different intensities. The second set was carried on with another apparatus, using the lowest intensity of light, 0.001 candle-metre. A third set of experiments, devised to check any disagreement between the results of the other two sets, due to a difference in the apparatus, was carried on with both pieces of apparatus, using the same light intensity for each.

The first apparatus (Fig. 1), which may be called the high-intensity apparatus, was essentially the same as that used by Parker and Arkin (:01, pp. 151–152). It consisted of a dark chamber (*C*) illuminated only through a horizontal oblong opening by a distant incandescent electric lamp (*B*). A glass jar (*A*), with parallel sides 6.25 cm. apart, and containing water, was placed immediately in front of the lamp to cut off heat. Within the dark chamber, directly in the path of the beam of light coming through the oblong opening, was a glass plate (*G*), suspended so that it was horizontal and could be easily rotated about its vertical axis. The glass was covered with wet filter paper, which was frequently changed. The light intensity is given for each experiment in candle-metres, and is the intensity of the beam of light at the centre of the plate. In all cases, the candle-power of the incandescent lamp was determined by a Lummer-Brodhun photometer.

In the first set of experiments, for each of the eleven light intensities, twenty fresh worms were used. Each worm was headed across the filter paper on the glass plate in a direction at right angles to the rays of light. As the worm crept, the plate was rotated, so that the axis of the worm was kept constantly at right angles to the

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direction of the light. Ten readings were taken on each worm; five with the light falling on the worm's right side, and five with the light on the left side. Thus, for each intensity, there were two hundred readings. As is well known, the worm creeps by projecting the head forward, and then drawing the body toward the head. When the worm projected the head in a line with the axis of the posterior part of the body, the reaction was called indifferent (o); when the head was moved to one side, if it was toward the light, the reaction was called positive (+); if away from the light, negative (—). For each of the eleven intensities, there was a total excess of negative over positive reactions. By subtracting the positive from the negative

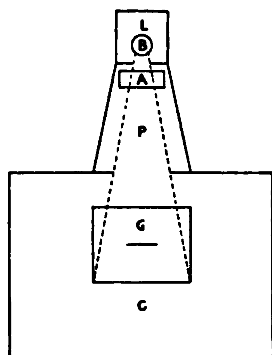


FIGURE 1. — Ground plan of high-intensity apparatus. *A*, glass vessel containing water to intercept heat rays; *B*, incandescent electric lamp; *C*, dark-chamber; *G*, glass plate covered with wet filter paper upon which the worms were made to creep; *L*, lantern; *P*, light-proof passage used by Parker and Arkin, but omitted in these experiments because the apparatus was set up in a dark-room with blackened wall.

reactions, the number of negative reactions due to the directive influence of the light was obtained; for, since the positive reactions are not caused by the light, but by other influences, such as irregularities of the surface over which the worm is creeping, etc., it is clear that an equal number of the negative reactions are likely to be caused by these other influences, and it is, therefore, necessary to subtract the number of these from the total number of negative reactions, to get the real number of such reactions due to light only.

Table I gives the result of the first set of experiments. The light intensities in candle-metres are given in the first column on the left. In the next two groups of columns are given, for the right and left sides respectively, the number of times the worm moved indifferently (o), negatively (—), and positively (+), and the excess of negative over positive reactions. In the third group, the total reactions of the worm for both sides are given.

From an inspection of the third group of the table, it appears that the maximum number of reactions to be attributed to the directive

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influence of the light is found, not in the most intense light, but in the third intensity used, 48 candle-metres. From this intensity there is a rather constant decrease in the amount of this excess, corresponding with a decrease of light intensity. The lowest intensity used with this apparatus, 0.012 candle-metre, shows an excess of only 6 negative reactions out of a total of 200. There is also a less regular increase in the number of positive reactions, as the light becomes less intense.

TABLE I.

REACTION OF ALLOLOBOPHORA TO LIGHT OF DIFFERENT INTENSITIES.

| Light intensity in candle-metres. | Reactions with light on right side of worm. | | | | Reactions with light on left side of worm. | | | | Total reactions of worm. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | − | + | Excess of − over +. | 0 | − | + | Excess of − over +. | 0 | − | + | Excess of − over +. |
| 192 | 30 | 58 | 12 | 46 | 35 | 51 | 14 | 37 | 65 | 109 | 26 | 83 |
| 90 | 42 | 53 | 5 | 48 | 51 | 42 | 7 | 35 | 93 | 95 | 12 | 83 |
| 48 | 36 | 62 | 2 | 60 | 34 | 62 | 4 | 58 | 70 | 124 | 6 | 118 |
| 31 | 47 | 46 | 7 | 39 | 39 | 56 | 5 | 51 | 86 | 102 | 12 | 90 |
| 12 | 43 | 53 | 4 | 49 | 46 | 48 | 6 | 42 | 89 | 101 | 10 | 91 |
| 5 | 52 | 43 | 5 | 38 | 47 | 46 | 7 | 39 | 99 | 89 | 12 | 77 |
| 1 | 62 | 30 | 8 | 22 | 53 | 37 | 10 | 27 | 115 | 67 | 18 | 49 |
| 0.128 | 44 | 36 | 20 | 16 | 50 | 31 | 19 | 12 | 94 | 67 | 39 | 28 |
| 0 050 | 40 | 35 | 25 | 10 | 32 | 41 | 27 | 14 | 72 | 76 | 52 | 24 |
| 0 020 | 40 | 32 | 28 | 4 | 44 | 31 | 25 | 6 | 84 | 63 | 53 | 10 |
| 0.012 | 49 | 26 | 25 | 1 | 51 | 27 | 22 | 5 | 100 | 53 | 47 | 6 |

The record for negative light reactions (26 per cent) for the single intensity of about 3.6 candle-metres obtained by Parker and Arkin finds an appropriate place in this table, in that, falling between 5 candle-metres and 1 candle-metre, its negative light reaction (26 per cent) also falls between those of 5 (38.5 per cent) and of 1 (24.5 per cent), though the position is not one that yields smooth results. The fact that the number of negative light reactions varies with the intensity makes it highly probable that the difference between the records of Parker and Arkin, and those of Smith, are to be explained as due to difference of intensity. Judged from the series

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exhibited in Table I, the intensity of light used by Smith was not far from 5 candle-metres, a condition easily obtainable from diffuse daylight.

The decrease in the excess of negative over positive reactions corresponding with a decrease of light intensities suggested that if the light were still further decreased, the negative light reactions might entirely disappear, or even be replaced by positive ones. To test this, the first piece of apparatus could not be used, because at intensities of light lower than 0.01 candle-metre it was found impossible to follow with the eye the movements of the worm. To obviate this difficulty, the second piece of apparatus was devised. This apparatus (Fig. 2), which may be called the low-intensity apparatus, consisted of two dark chambers in contact with each other, shown in plan in the figure. In the first chamber (*A*), a vertical white screen (*CD*) was placed in a diagonal position, so that it reflected light from an electric incandescent lamp (*E*) through a small aperture (*F*) between the two chambers. This reflected light entered the second chamber (*B*), and there passed over a horizontal glass plate (*G*). It was found that when the relations of the light, screen, and plate were such as are given in the diagram, with an incandescent lamp of 8.25 candle-power, and a circular aperture of 1 mm. diameter, an illumination of 0.0011 candle-metre was obtained at the centre of the plate. Wet filter paper was used on the plate, and two thick, narrow strips of glass (*HH*), like those employed by Smith (:02), were placed on the filter paper in parallel positions, with only enough space between them to allow the worm to creep. This narrow path between the two strips was in a direction at right angles to the central rays of light which came through the aperture. After starting the worm in this path, the chambers were closed so as to exclude all outside light. Time enough was given to allow the worm to creep into the field beyond the two strips of glass; chamber *B* was then opened, and the position of the worm noted. If the worm was headed toward the light, it was called a positive reaction; if away from the light, a negative one; and if the worm had moved without changing its original direction, an indifferent one.

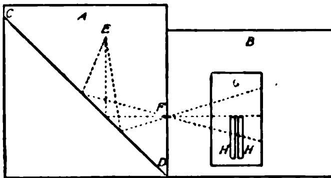

FIGURE 2.

Twenty fresh worms were used as before, and for each worm two sets of readings were made. In one set, the worm was illuminated

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by the beam of light of 0.0011 candle-metre intensity; in the other set, the aperture was closed, that is, the worm was in total darkness.

TABLE II.

REACTIONS OF ALLOLOBOPHORA TO LIGHT OF 0.0011 CANDLE-METRE, AND IN DARKNESS.

| Number of worm. | 0.0011 candle-metre. | | | | Darkness. | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | + | – | Excess of + over –. | 0 | + | – | Excess of + or of –. |
| 1 | 0 | 7 | 3 | 4 | 1 | 2 | 7 | 5– |
| 2 | 0 | 8 | 2 | 6 | 0 | 5 | 5 | 0 |
| 3 | 0 | 6 | 4 | 2 | 0 | 6 | 4 | 2+ |
| 4 | 0 | 6 | 4 | 2 | 0 | 7 | 3 | 4+ |
| 5 | 0 | 6 | 4 | 2 | 0 | 7 | 3 | 4+ |
| 6 | 0 | 5 | 5 | 0 | 0 | 5 | 5 | 0 |
| 7 | 0 | 7 | 3 | 4 | 2 | 5 | 3 | 2+ |
| 8 | 0 | 5 | 5 | 0 | 1 | 5 | 4 | 1+ |
| 9 | 1 | 5 | 4 | 1 | 0 | 4 | 6 | 2– |
| 10 | 0 | 8 | 2 | 6 | 0 | 4 | 6 | 2– |
| 11 | 0 | 8 | 2 | 6 | 0 | 6 | 4 | 2+ |
| 12 | 0 | 6 | 4 | 2 | 0 | 7 | 3 | 4+ |
| 13 | 0 | 8 | 2 | 6 | 1 | 4 | 5 | 1– |
| 14 | 0 | 8 | 2 | 6 | 0 | 4 | 6 | 2– |
| 15 | 0 | 6 | 4 | 2 | 1 | 5 | 4 | 1+ |
| 16 | 1 | 8 | 1 | 7 | 0 | 6 | 4 | 2+ |
| 17 | 0 | 8 | 2 | 6 | 1 | 4 | 5 | 1– |
| 18 | 0 | 7 | 3 | 4 | 0 | 3 | 7 | 4– |
| 19 | 0 | 9 | 1 | 8 | 0 | 6 | 4 | 2+ |
| 20 | 1 | 7 | 2 | 5 | 0 | 5 | 5 | 0 |
| Totals | 3 | 138 | 59 | 79+ | 7 | 100 | 93 | 7+ |

In each set, ten readings were made on each worm, five with the worm's right side toward the aperture, which in one set was open and in the other closed, and five with the left side toward the aperture.

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Table II gives the reactions of the twenty worms. The readings of the right and left sides of the worm have been combined. In the first column on the left is given the number of the worm. In the left-hand group of columns are given the reactions of the worm in light of 0.0011 candle-metre; and in the right-hand group are given the reactions in darkness. At the bottom, the total reactions for the twenty worms are recorded (see Table II, page 31).

It appears, first, that there is a great reduction in the number of indifferent movements over the number obtained with the first piece of apparatus. This is explained by the fact that in the second apparatus the slightest deviation of the worm from the indifferent path, which was marked by a pencil line on the filter paper, could be observed; and thus many reactions which would have been recorded as indifferent in the first apparatus were here shown to be either positive or negative. Of the reactions of the worms in total darkness, in all 200, 7 were indifferent, 100 were toward the aperture (which was now closed), and 93 were away from it. The 200 reactions of the same worms in light of 0.0011 candle-metre at the plate, were as follows: 3 were indifferent, 138 were toward the light, and 59 were away from the light, there being an excess of 79 positive reactions. Moreover, there was never found an excess of negative over positive movements in the case of any worm. This clearly points to the conclusion that light of low intensity induces positive phototropic reactions.

To ascertain whether the results obtained by the high-intensity and by the low-intensity apparatus were fairly comparable, a third set of experiments was tried, in which worms were tested at the same light intensity (1 candle-metre) in both pieces of apparatus. Twenty fresh worms were used, each one first in one apparatus, and then in the other. In each apparatus, ten readings were made, five with the light on the right side, and five with it on the left side of the worm.

Table III shows the result of this set of experiments. The figures given are the totals for the twenty worms, right and left sides combined (see Table III, page 33).

A much larger number of indifferent movements were obtained on the high-intensity apparatus, but the total excess of negative over positive movements is so nearly alike for each (25 per cent and 29 per cent), that one may assume that the records of the two sets of experiments are fairly comparable, and therefore, from the observations recorded in Tables I and II, draw the conclusion that Allolo-

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bophora fœtida is negative to light intensities between 192 and 0.012 candle-metres, and positive at 0.0011 candle-metre.

The results of these experiments are in harmony with certain daily habits of earthworms. During the daytime, while the light is of

TABLE III.

| Direction of reactions. | 0 | – | + | Excess of – over +. |
|---|---|---|---|---|
| High-intensity apparatus . . | 96 | 77 | 27 | 50 |
| Low-intensity apparatus . . | 4 | 127 | 69 | 58 |

relatively high intensity, these animals retreat into their burrows because of negative phototropism. At night-time, however, they emerge from their burrows, not because of the absence of light, but by reason of their positive phototropism to light of low intensity, for even in the darkest night there is faint light.

## Conclusions.

1. Allolobophora fœtida is *negatively* phototropic toward the light from electric incandescent lamps varying in intensity from 192 candle-metres to 0.012 candle-metre; the percentage of negative head-movements referable to lights of different intensities are as follows: 41.5 per cent (192 cm.), 41.5 per cent (90 cm.), 59 per cent (48 cm.), 45 per cent (31 cm.), 45.5 per cent (12 cm.), 38.5 per cent (5 cm.), 24.5 per cent (1 cm.), 14 per cent (0.128 cm.), 12 per cent (0.050 cm.), 5 per cent (0.020 cm.), and 3 per cent (0.012 cm.).

2. A. fœtida is *positively* phototropic toward an electric incandescent lamp of 0.0011 candle-metre intensity.

3. Earthworms retreat into their burrows during daytime because of their negative phototropism. They emerge at night not so much because of darkness as because of their positive phototropism for faint light.

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# AN EXPERIMENTAL STUDY OF THE SUGAR CONTENT AND EXTRAVASCULAR COAGULATION OF THE BLOOD AFTER ADMINISTRATION OF ADRENALIN.

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## INTRODUCTION.

EARLY in 1902 the discovery was made by Herter and Richards[2] that the injection of solutions of adrenalin chloride into the peritoneal cavity of dogs was followed by an intense though transient glycosuria. It was also found that the application of adrenalin solution directly to the surface of the pancreas produced a similar effect. As a result of a number of experiments in this direction, the suggestion was offered that this form of glycosuria was in reality of pancreatic origin.

In extending these observations, Herter and Wakeman[3] have found that the power of adrenalin to produce glycosuria, when applied to the pancreas, is not specific but is shared with various substances. The number of such substances is comparatively large, and apparently the only quality common to the series is a reducing activity. A seeming exception to this rule was found in potassium cyanide. When solutions of this substance were applied to the pancreas in amounts far too small to produce general toxic symptoms, glycosuria resulted. This substance, like hydrocyanic acid, while it has no reducing power, exerts a specific action on the animal cells in preventing them from absorbing oxygen.[4] It is natural to suppose that, in the absence of the normal amount of oxygen in the cell, an excess

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[2] HERTER and RICHARDS: The medical news, 1902, lxxx, p. 201.

[3] HERTER and WAKEMAN: Virchow's Archiv für pathologische Anatomie und Physiologie und für klinische Medicin, 1902, clxix, p. 479; HERTER: The medical news, 1902, lxxx. p. 867.

[4] GEPPERT: Zeitschrift für klinische Medicin, 1889, xv, p. 208; *Ibid.*, p. 307.



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of reducing substances may be formed. It is possible that these substances may act in a manner comparable to those of the above-mentioned series in bringing about the excretion of sugar. From the facts brought out by their observations in this regard, Herter and Wakeman are inclined to attribute the production of glycosuria upon the application of adrenalin and other substances to the pancreas to a toxic action on the cells of that gland which is closely connected with the power of reduction.

If this view of the matter is correct, an important relationship suggests itself between this form of experimental glycosuria and conditions in the human organism which may give rise to an excretion of sugar. The fact that many organs of the body may form reducing substances capable of easy oxidation which may reach the pancreas in the blood stream, carries with it the possibility that, if the normal balance between the amount of these substances and the oxidizing power of the pancreas be disturbed, the production of glycosuria may occur.

Concerning the mechanism through which adrenalin brings about the excretion of sugar no positive statements can as yet be made. The work of Minkowski [1] and his followers, which has furnished the basis of the belief in the existence of an internal secretion of the pancreas exercising a controlling influence on carbohydrate metabolism, justifies an assumption that the sugar elimination is the result of an alteration in the nature, activity, or amount of this secretion. The glycosuria-producing effect of injury of certain parts of the central nervous system,[2] and the increase in sugar formation in the liver which follows stimulation of the cœliac plexus [3] or of the vagus nerve,[4] may lead to the supposition that adrenalin glycosuria results from the action of a nervous mechanism. Finally, it is known that under the abnormal conditions which follow the injection of phlorhizin [5] or chromic acid,[6] glycosuria may occur, owing to an increase in the permeability of the kidney cells. The possibility that adrenalin

[1] MINKOWSKI: Untersuchungen über den Diabetes Mellitus nach Extirpation des Pancreas, Leipzig, 1893.

[2] CL. BERNARD: Leçons sur la physiologie et la pathologie du system nerveux, Paris, 1858, i, p. 401.

[3] A. and E. CAVAZZANI: Centralblatt für Physiologie, 1894, viii, p. 33.

[4] LEVENE: Centralblatt für Physiologie, 1894, viii, p. 337.

[5] v. MERING: Zeitschrift für klinische Medicin, 1889, xvi, p. 431.

[6] KOSSA: Archiv für die gesammte Physiologie, 1901–1902, lxxxviii, p. 627.

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glycosuria is the immediate result of changes in the kidney has not yet been excluded.

Whatever may be the manner by which the effects of adrenalin are brought about, it is probable that the mechanism involved is one which is active, though in a different degree, under normal conditions. A determination of the identity of the mechanism is therefore of importance, not only in explaining the phenomenon in question, but also from the fact that it may throw light on some of the processes connected with the normal metabolism of carbohydrate within the organism.

Before such a determination can be made, however, a more accurate knowledge of the internal conditions antecedent to the excretion of sugar is necessary. With this end in view we have made a somewhat detailed study of the sugar in the blood, after intraperitoneal injection of adrenalin, as well as after application of that substance to the pancreas.

## Sugar Content and Coagulation of Arterial Blood after Treatment with Adrenalin.

It has long been known that the glycosuria produced by extirpation of the pancreas,[1] puncture of the floor of the fourth ventricle,[2] and poisoning with certain substances, such as carbon monoxide,[3] is the immediate result of an increased accumulation of sugar in the blood. On the other hand, injections of phlorhizin[4] are followed by the excretion of sugar due to the effect on the kidney. In the latter case the percentage of sugar in the blood never rises above normal, and may even fall below that amount. To determine in which class adrenalin glycosuria belongs, we have made a number of determinations of the sugar content of the blood of dogs which had been subjected to treatment with adrenalin. In this series also we have attempted to ascertain the rapidity with which this substance acts, and the course and duration of its influence.

**Method of collection and analysis of blood.** — Healthy, well-nourished dogs were anæsthetized with pure ether, a cannula introduced into a femoral artery, and a portion of blood taken. The solution of adrena-

[1] Minkowski: *Loc. cit.*

[2] Cl. Bernard: *Loc. cit.*

[3] Senff: Ueber den Diabetes nach der Kohlenoxydathmung, Inaugural dissertion, Dorpat, 1869.

[4] v. Mering: *Loc. cit.*

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lin chloride[1] was then injected by a hypodermic syringe into the peritoneal cavity or, after an incision through the abdominal wall, was painted on the surface of the pancreas with a soft brush. Portions of blood were then drawn from the femoral artery at various intervals.

Having in mind the possible production of glycosuria by means of anæsthetics,[2] as well as by asphyxia,[3] care was taken to keep the anæsthesia as light and as constant as possible. Moreover, we believe that this factor may be left out of account in these experiments, since the control portion of blood, taken before adrenalin treatment, was collected under the same conditions of anæsthesia as the subsequent portions which are compared with it.

The portions of blood were analyzed according to the following procedure. The blood was drawn directly into a beaker containing a solution of phosphotungstic acid in dilute hydrochloric acid.[4] The beaker was counterpoised on a balance and the blood weighed immediately after its withdrawal from the artery. On boiling this mixture the blood proteids are precipitated in a granular form leaving a water-clear fluid free from proteid. The precipitate was washed thoroughly with hot water, a process which is rendered easy by its porosity and its friable character. The combined filtrate and washings were nearly neutralized with sodium hydroxide and evaporated to small bulk on the water bath. The evaporated residue was made up to known volume (50–100 c.c.) with water, and was filtered. The reducing power of this solution was determined by the Allihn method. The results were calculated in terms of dextrose from the weight of the metallic copper. The figures given represent the averages of closely agreeing duplicates.

**Method of determining coagulation.** — In one of our early experiments we noticed that a portion of blood drawn for the purpose of rinsing the cannula clotted very rapidly. As a result of this observation, in a number of later experiments we have taken separate por-

[1] In all the experiments outlined in this paper, the adrenalin chloride solution ($\frac{1}{1000}$) prepared by Parke, Davis, & Co., by the method of Takamine, was used.

[2] CUSHNY: Pharmacology and Therapeutics or the action of drugs, 1899, p. 160.

[3] DASTRE: Comptes rendus des séances de l'academie des sciences, 1879, lxxxix, p. 669.

[4] This solution contained 70 gms. of phosphotungstic acid and 20 c.c. of hydrochloric acid, sp. gr. 1.20, in a litre. About 5 c.c. are sufficient to completely precipitate the proteids in 1 gm. of blood.

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tions of blood to be tested regarding this point. The amount drawn in each case was 2 c.c., collected in a graduated cylinder of 5 c.c. capacity. The time which elapsed between the collection of the blood and the time at which the cylinder could be inverted without loss of its contents, was noted as the time of the coagulation of the blood.

The results of our determinations are given in Table I, pages 40, 41.

These experiments show unmistakably that the administration of adrenalin chloride either by intraperitoneal injection or by painting it upon the pancreas is followed by a marked increase of sugar in the blood. The increase is very noticeable within the first five minutes after the application and reaches its maximum within three hours. A very gradual fall then ensues, which may continue until the percentage of sugar becomes subnormal (Exp. 2). In a dog recently fed (Exp. 1), the blood sugar may be double the normal quantity fourteen hours after the injection. A marked rise occurred in the case of a dog (Exp. 7) which had been starved for six days. In Experiment 8 a fatal dose of adrenalin was given. A slight increase in the sugar content of the blood occurred shortly after. One minute before death ensued, twenty-four hours after injection, the percentage of sugar was approximately normal.

Simultaneously with the production of hyperglycæmia, an effect on the coagulability of the blood is observed. In every case, without exception, the time of coagulation is lessened after adrenalin is given. This diminution is equal in some cases to four-fifths of the coagulation time of the control.

Arthus[1] has shown that the time of coagulation decreases if the blood is allowed to come in contact with blood already clotted or with an exposed tissue surface. Special care has been taken therefore in these experiments to remove the clot from the cannula before each collection. Furthermore the portion for the coagulation test was collected just after that for sugar analysis, a circumstance which insures the rinsing of the cannula.

The recent observation[2] by the same author that the mere withdrawal of large amounts of blood from the body hastens the coagulation of subsequent portions, raises the question whether the results which we have observed may have been due to loss of blood alone. To test this point, a control experiment was made in which the

[1] ARTHUS: Journal de physiologie et de la pathologie générale, 1902, iv, p. 283.
[2] ARTHUS: *Ibid.*, p. 273.

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TABLE I.

[illegible]ENT AND COAGULATION OF ARTERIAL BLOOD AFTER TREATMENT WITH ADRENALIN.

| No. of experiment. | Weight of dog. Kilo. | Time since last fed. | Amount of blood withdrawn. Grams. | Time when taken.* | Sugar. Per cent. | Time of coagulation. min. sec. | Remarks. |
|---|---|---|---|---|---|---|---|
| 1 | 8.5 | 12 hrs. | 29.0 | 9.30 P. M.: normal | 0.16 | | Etherized at 9.15 P. M.; 8 c.c. adrenalin chloride solution ($\frac{1}{1000}$) injected intraperitoneally at 9.35 P. M.; anæsthesia continued till 12.30 A. M.; again etherized on following morn. at 11.30. |
| | | | 30.0 | 10.35 P. M.: 1 hr. after injection | 0.42 | | |
| | | | 25.0 | 11.30 P. M.: 2 hrs. " " | 0.56 | | |
| | | | 24.0 | 12.30 A. M.: 3 " " " | 0.52 | | |
| | | | 33.75 | 11.45 A. M.: 14 " " " | 0.37 | | |
| 2 | 7.6 | 32 hrs. | 29.0 | 5.44 P. M.: normal | 0.258 | 4 00 | Etherized at 5.30 P. M.; 8 c.c. of adrenalin chloride solution ($\frac{1}{1000}$) injected intraperitoneally at 5.47 P. M.; anæsthesia continued till 7.00 P. M.; again etherized at 10.30 P. M., also at 10.30 A. M. next morning. |
| | | | 15.0 | 5.52 P. M.: 5 min. after injection | 0.357 | 2 15 | |
| | | | 11.5 | 5.57 P. M.: 10 " " " | 0.400 | 2 00 | |
| | | | 10.7 | 6.20 P. M.: 33 " " " | 0.437 | 2 20 | |
| | | | 11.8 | 6.47 P. M.: 1 hr. " " | 0.430 | 4 10 | |
| | | | 10.8 | 10.35 P. M.: 5 hrs. " " | 0.307 | 3 05 | |
| | | | 27.3 | 10.38 A. M.: 17 " " " | 0.099 | 3 00 | |
| 3 | 12.0 | 48 hrs. | 22.4 | 3.00 P. M.: normal | 0.112 | | Etherization continued throughout experiment; 3 c.c. of adrenalin chloride solution ($\frac{1}{1000}$) applied to pancreas with a brush at 3.08 P. M. |
| | | | 21.5 | 3.15 P. M.: 7 min. after painting | 0.182 | | |
| | | | 22.5 | 3.23 P. M.: 15 " " " | 0.178 | | |
| | | | 19.7 | 3.38 P. M.: 30 " " " | 0.188 | | |
| | | | 20.0 | 4.08 P. M.: 1 hr. " " | 0.204 | | |
| | | | 21.4 | 5.06 P. M.: 2 hrs. " " | 0.214 | | |
| | | | 20.8 | 6.00 P. M.: 3 " " " | 0.165 | | |
| 4 | 7.1 | 48 hrs. | 21.9 | 7.55 P. M.: normal | 0.173 | | Etherized from 7.45 till end of experiment; 4 c.c. adrenalin chloride solution ($\frac{1}{1000}$) painted on surface of pancreas at 8.12 P. M. |
| | | | 27.3 | 8.17 P. M.: 5 min. after painting | .... | | |
| | | | 21.1 | 8.27 P. M.: 15 " " " | 0.277 | | |
| | | | 20.4 | 8.42 P. M.: 30 " " " | 0.237 | | |
| | | | 21.8 | 9.12 P. M.: 1 hr. after painting | 0.291 | | |
| | | | 21.3 | 10.12 P. M.: 2 hrs. " " | 0.256 | | |

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| | | | | | | min. | sec. | |
|---|---|---|---|---|---|---|---|---|
| 5 | 13.2 | 24 hrs. | 19.9 | 10.45 A.M.: normal | 0.239 | 5 | 20 | Ether given throughout the experiment; 3 c.c. of adrenalin chloride ($\frac{1}{1000}$) applied to pancreas at 10.55 A.M. |
| | | | 23.8 | 11.00 A.M.: 5 min. after painting | 0.291 | 3 | 00 | |
| | | | 25.8 | 11.10 A.M.: 15 " " " | 0.354 | 1 | 57 | |
| | | | 19.4 | 11.25 A.M.: 30 " " " | 0.388 | 2 | 07 | |
| | | | 20.3 | 11.54 A.M.: 1 hr. " " | 0.433 | 1 | 12 | |
| | | | 21.2 | 12.54 P.M.: 2 hrs. " " | 0.477 | 1 | 35 | |
| | | | 19.3 | 1.54 P.M.: 3 " " " | 0.519 | 1 | 15 | |
| | | | 20.0 | 2.55 P.M.: 4 " " " | 0.465 | 0 | 50 | |
| 6 | 7.0 | 24 hrs. | 17.9 | 4.52 P.M.: normal | 0.131 | 6 | 50 | Ether given throughout experiment; 2 c.c. of adrenalin chloride solution ($\frac{1}{1000}$) applied to pancreas at 5.00 P.M. |
| | | | 17.0 | 5.05 P.M.: 5 min. after painting | 0.205 | 3 | 05 | |
| | | | 20.5 | 5.15 P.M.: 15 " " " | 0.217 | 1 | 40 | |
| | | | 19.0 | 5.30 P.M.: 30 " " " | 0.264 | 1 | 20 | |
| | | | 18.3 | 6.00 P.M.: 1 hr. " " | 0.315 | 1 | 47 | |
| | | | 17.0 | 7.00 P.M.: 2 hrs. " " | 0.371 | 1 | 40 | |
| 7 | 7.4 | 6 days | 17.0 | 3.34 P.M.: normal | 0.154 | 2 | 00 | Ether given throughout experiment; 3 c.c. of adrenalin solution applied to pancreas at 3.43 P.M. |
| | | | 17.6 | 3.49 P.M.: 6 min. after painting | 0.192 | 2 | 25 | |
| | | | 18.2 | 4.01 P.M.: 18 " " " | 0.173 | 2 | 40 | |
| | | | 17.0 | 4.22 P.M.: 29 " " " | 0.191 | 2 | 05 | |
| | | | 16.5 | 4.58 P.M.: 1 hr. 15 min. aft. painting | 0.206 | 2 | 15 | |
| | | | 17.7 | 5.43 P.M.: 2 hrs. after painting | 0.143 | 1 | 55 | |
| | | | 18.5 | 6.37 P.M.: 3 " " " | 0.169 | 1 | 16 | |
| 8 | 13.7 | 5 days | 14.9 | 9.23 P.M.: normal | 0.138 | 4 | 30 | Anæsthetized with mixt. of chloroform and ether; 12 c.c. of adrenalin chloride sol. ($\frac{1}{1000}$) injected intraperitoneally at 9.33.30 P.M.; kept under ether till 11.15 P.M.; etherized at 10.30 following morning; animal very much prostrated; no ether given at night; characteristic bloody mucous passed in fæces; died 1 min. after last portion of blood was collected. |
| | | | 15.6 | 9.35 P.M.: 1 min. 30 sec aft. injection | 0.104 | 1 | 20 | |
| | | | 11.3 | 10.18 P.M.: 45 min. after injection | 0.095 | 2 | 20 | |
| | | | 10.4 | 11.03 P.M.: 1 hr. 30 min. aft. injection | 0.199 | 3 | 15 | |
| | | | 19.0 | 10.31 A.M.: 13 hrs. after injection | 0.058 | 2 | 40 | |
| | | | 26.5 | 9.38 P.M.: 24 " " " | 0.102 | 5 | 30 | |

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amount of blood taken was very small. The details of the experiment are as follows:

A small dog, 5.3 kilos in weight, which had received no food for twenty-eight hours previous to the experiment, was etherized and a cannula introduced into the femoral artery. 2 c.c. of blood collected at 2.34 P. M., coagulated in 5 min. 50 sec. An incision was made through the abdominal wall and 2 c.c. of adrenalin solution applied to the pancreas with a soft brush at 2.43 P. M. Subsequent samples of blood (2 c.c. each) coagulated as follows:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 min. | after | adrenalin | application | (2.48 P. M.) | coagulated in | 1 min. | 45 secs. |
| 16 " | " | " | " | (2 59 P. M.) | " | 2 " | 0 " |
| 33 " | " | " | " | (3.16 P. M.) | " | 2 " | 32 " |
| 1 hr. | " | " | " | (3.45 P. M.) | " | 3 " | 10 " |

Other control experiments in which large amounts of blood were removed, and no treatment with adrenalin given, show no such marked changes as are seen in the observations in Table I.

## The Source of the Excess of Sugar in the Blood in Adrenalin Glycosuria, as Indicated by Comparative Analysis of Blood Collected Simultaneously from the Portal and Hepatic Veins and the Femoral Artery.

The results just detailed show clearly that the phenomenon of adrenalin glycosuria is due to an increase of sugar in the blood. The source of this excess of sugar is of great importance in determining the mechanism by which this effect is brought about. We have endeavored to trace the source of the sugar by means of analysis of blood taken simultaneously from the portal and hepatic veins and from the femoral artery. In these experiments it was necessary to collect successive portions of blood at various intervals from the same blood-vessel without interfering with the normal circulation in that vessel. The methods which have hitherto been devised for the collection of portal and hepatic blood appeared to be inadequate for our purpose, as well as somewhat difficult and uncertain of application.[1] We have therefore made use of an original method, the description of which follows. A cannula of special design[2] (see accompanying diagram) is the essential feature of the method.

[1] For description of older methods, see Seegen: Die Zuckerbildung im Thierkörper, 2 Auflage, Berlin, 1900, p. 66.

[2] The special cannulas used in these experiments were made for us very skilfully by Mr. John T. Hoyt of the Department of Physiology in this institution.

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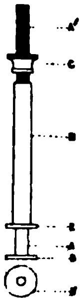
**General method.** — The method of fixing the cannula into a vein is as follows: The vessel is carefully exposed, and the outer connective tissue sheath dissected away. Loose ligatures are passed about the vessel at each end of the cleared portion, which should be about 2 cm. in length. Before introducing the cannula, its parts should be so arranged that the flange of the outer tube is separated by a space of about 0.5 cm. from that of the inner tube. The brass rod should be in position, totally filling up the bore of the inner tube. The ligatures about the vessel are then tightened momentarily by an assistant, and a longitudinal slit made in the wall of the vessel between them. This slit should be a trifle shorter than the diameter of the flange. The flange of the inner tube is then introduced into the interior of the vessel through the slit, the outer tube pressed down till the



EXPLANATION OF DIAGRAM. — The cannula consists of three parts, viz.: an inner tube (*A*), an outer tube (*B*), and a nut (*C*). The inner tube is about 6.5 cm. in length. Its outer diameter is 4 mm., its inner diameter, 2 mm. On it a screw thread (*A′*) is cut, extending for 3.5 cm. from one end. The other end is provided with a flange (*D*) 9 mm. in diameter. The end view of this flange is shown at *D′*. The outer tube (*B*), 3.5 cm. long, fitting closely over the inner tube, terminates in a flange (*E*) similar to the one on the latter. By means of the nut the two tubes may be held in such a position that their flanges are in close contact. All parts of the instrument are made of brass. The cannula is also provided with a brass rod (not shown in the diagram) about 28 cm. long, of such a thickness that it fits closely into the bore of the inner tube. A small shoulder brazed on the rod at the proper point insures the complete occlusion of the cannula when desired.

wall of the vessel is held tightly between the two flanges, and the nut screwed down so that the hold is retained. The ligatures are then loosened and the normal circulation is resumed. With a little practice the operation can be accomplished with no loss of blood and an interruption of the circulation of only thirty to forty seconds. On connecting a rubber tube with the inner tube of the cannula and removing the brass rod, blood can be withdrawn at pleasure.

In our experiments to determine the sugar content of the blood flowing to and from the liver, we have introduced cannulas of this type into the portal vein at its juncture with the pancreatico-duodenalis, and into one of the larger hepatic veins at a point between the liver and the diaphragm.

In order to expose the vessels, a transverse cut was made through the abdominal wall, following the curvature of the free border of the ribs and extending for about three inches on each side of a point on the median line just below the xyphoid cartilage. Bleeding was prevented by ligaturing the vessels which it was necessary to cut. The abdominal organs were protected from exposure by cloths moistened with warm saline solution. The time necessary for the operation and the introduction of the cannulas into both veins usually amounted to about one hour.

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In drawing blood from the hepatic vein it was necessary to use a suction pump to overcome the negative pressure, which is very manifest in the venous circulation at this point. For this purpose, the beaker containing the phosphotungstic mixture, previously weighed, was placed under a small bell-jar which was connected by its lower opening with a suction pump. A small glass tube inserted through the rubber stopper which closed the upper opening of the bell-jar, and terminating at a point directly over the beaker, served to conduct the blood into the precipitating fluid. On connecting the cannula in the hepatic vein with the glass tube, and applying suction, the estimated amount of blood is easily obtained.

For collecting arterial blood, a glass cannula of the ordinary type was introduced into the femoral artery.

The order of procedure in obtaining the portions of blood simultaneously was as follows: The beakers which were to hold the femoral and portal blood were counterpoised on balances placed at the side of the operating table. Small glass tubes were clamped in a position to lead the blood into them. The beaker for collection of hepatic blood was placed under the bell-jar arranged as described above. The brass rods were removed from the cannulas in the portal and hepatic veins, and connection with the proper glass tubes made, passage of blood being prevented by clamping the rubber tubes. The connection between the cannula in the femoral and the third glass tube was made. At a given signal the clamps were removed and the blood collected. The brass rods were immediately replaced in the cannulas, and the portions of blood weighed. The time necessary for collection of all three portions of blood has seldom amounted to more than ten or fifteen seconds.

The analysis of the blood was carried out in a manner exactly similar to that previously described.

By means of these methods we have succeeded in obtaining blood simultaneously from the three vessels mentioned, both before and after the application of adrenalin chloride to the surface of the pancreas. The results of sugar determination are given in the table on page 45.

The results of Experiments 1, 2, 3, and 6 present, we believe, a true picture of the events in this connection succeeding the application of adrenalin to the pancreas. The samples of blood taken before treatment with adrenalin agree fairly closely in their sugar content. On the ground of these figures it cannot be said that the amount of sugar in the blood issuing from the liver is greater than that of the femoral artery or portal vein. After treatment with adrenalin, however, the relations are changed. In Experiment 3, four minutes after

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TABLE II.—SUGAR CONTENT OF BLOOD COLLECTED SIMULTANEOUSLY FROM THE HEPATIC AND PORTAL VEINS AND FEMORAL ARTERY BEFORE AND AFTER TREATMENT WITH ADRENALIN.

| No. of exp. | Weight Kilo. | Time after last fed Hrs. | Amount of blood from Femoral artery Grms. | Amount of blood from Portal vein Grms. | Amount of blood from Hepatic vein Grms. | Time of collection P.M. | Time of application of adrenalin P.M. | Time of collection compared with time of application | Sugar in blood from Femoral artery Per cent | Sugar in blood from Portal vein Per ct. | Sugar in blood from Hepatic vein Per ct. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 6 min. before | 0.10 | 0.10 | 0.08 | Chest opened[1] and artificial respiration employed. |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 30 min. after | 0.18 | 0.08 | 0.25 | |
| 2 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 16 min. before | 0.160 | 0.165 | 0.161 | At time of first collection of blood, pancreas and intestines normal in appearance. At second collection, pancreas somewhat congested, intestines somewhat cyanotic. At third drawing of blood, the pancreas was very much congested and small intestine very blue. |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 10 min. after | [illegible] | 0.197 | [illegible] | |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 11 " " | [illegible] | 0.218 | 0.316 | |
| 3 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 19 min. before | 0.150 | 0.160 | 0.146 | At time of first and second collections of blood, the pancreas and small intestines were normal in appearance. When the third and fourth series were taken, these organs were much congested. |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 4 min. after | 0.187 | 0.167 | 0.201 | |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | [illegible] " " | [illegible] | 0.157 | [illegible] | |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | [illegible] " " | 0.308 | 0.207 | 0.209 | |
| 4 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 14 min. before | 0.111 | 0.262 | 0.116 | At first drawing of blood, the pancreas and intestines were normal. At the second, the small intestine was congested and cyanotic. |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 27 min. after | 0.188 | 0.114 | 0.118 | |
| 5 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 10 min. before | [illegible] | [illegible] | 0.210 | Pancreas and intestines normal at time of first collections. Pancreas congested and small intestine congested and cyanotic at time of second. |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 21 min. after | [illegible] | 0.158 | [illegible] | |
| 6 | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 10 min. before | 0.201 | 0.181 | 0.208 | Pancreas and intestines normal in appearance at first collections. Pancreas congested and intestines congested and cyanotic at time of second. |
| | | | [illegible] | [illegible] | [illegible] | [illegible] | | 28 min. after | 0.308 | [illegible] | [illegible] | |

[1] In this experiment only was it found necessary to open the chest in order to insert the cannula into the hepatic vein.

the application of the substance to the gland the sugar content of the arterial blood rises 0.028 per cent, that of the portal blood remains practically the same, while the increase in reducing power of the blood emerging from the liver amounts to 0.065 per cent. The same relation, though on a higher plane, is apparent twenty-six minutes later. Sixty-six minutes after, the sugar percentage from all the vessels is approximately the same. Precisely similar results are obtained in Experiments 1 and 2, and in a lesser degree in Experiment 6. Judging from the results of these analyses then, a formation of sugar in the liver must be the cause, in part at least, of the increase of sugar in the blood.

Experiments 4 and 5 apparently form exceptions to this conclusion. It will be noticed, however, that the percentage of sugar in the samples taken before adrenalin treatment are abnormally high, especially in Experiment 4. It is possible that the mechanism which takes part in the production of adrenalin glycæmia has already been affected by the operative disturbance. It is not an unfair assumption that the additional impulse given by the application of adrenalin is on that account less effective and its result more transient. Consequently at the time of the collection of the second portions of blood the secretion of sugar is lessening. The same reasoning may hold good with regard to Experiment 5.

Comparison of the sugar content of the portal blood with that from the femoral artery and hepatic vein in Experiments 1, 2, 3, and 5 shows that the increase of sugar following treatment of the pancreas with adrenalin is least in the portal vein. While in the control samples the sugar percentage of the portal is as high or higher than that of the femoral or hepatic blood, after treatment with adrenalin, it is lower in every case. In this connection we would call attention to certain changes in the appearance of the organs of the abdomen. At the time of collection of the first samples of blood the appearance of the intestines and pancreas was normal. As the experiment proceeded, however, the pancreas became congested and the intestines cyanotic. The latter symptom is due, probably, to a partial obstruction of the circulation by the formation of a clot at the flange of the cannula. The effect of this partial obstruction is a partial asphyxia of the tissues. That the *relative* decrease in the sugar of the portal blood is dependent upon increased utilization within the tissues through which it passes there can be no doubt. Whether this consists in a mere increased oxidation of sugar, owing to the increased

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TABLE III.

SUGAR CONTENT OF BLOOD TAKEN SIMULTANEOUSLY FROM THE HEPATIC AND PORTAL VEINS AND THE FEMORAL ARTERY, TREATMENT WITH ADRENALIN BEING OMITTED.

| No of experiment. | Weight. | Time since fed. | Amount of blood from | | | Time of collection. | Sugar in blood from | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Kilo. | Hours. | Femoral artery. Grams. | Portal vein. Grams. | Hepatic vein. Grams. | | Femoral artery. Per cent. | Portal vein. Per cent. | Hepatic vein. Per cent. | |
| 1 | 13.6 | 22 | 14.5 | 13.1 | 16.4 | 4.45 P. M. | 0.236 | 0.213 | 0.210 | Appearance of the pancreas and intestines normal throughout experiment. |
| | | | 12.9 | 12.9 | 11.6 | 5.05 P. M. | 0.220 | 0.193 | 0.242 | |
| | | | 13.6 | 11.7 | 13.7 | 5.30 P. M. | 0.190 | 0.198 | 0.205 | |
| | | | 13.5 | 15.1 | 13.7 | 6.05 P. M. | 0.217 | 0.185 | 0.217 | |
| 2 | 7.7 | 20 | 12.0 | 14.2 | 13.8 | 4.24 P. M. | 0.174 | 0.160 | 0.166 | Intestines became somewhat blue at the time of second and third collections of blood. Pancreas normal. |
| | | | 14.3 | 13.5 | 16.7 | 4.46 P. M. | 0.183 | 0.179 | 0.188 | |
| | | | 15.8 | 11.9 | 13.2 | 5.16 P. M. | 0.194 | 0.173 | 0.174 | |
| 3 | 17.15 | 48 | 13.5 | 15.1 | 13.3 | 12.10 P. M. | 0.215 | 0.174 | 0.216 | Intestines somewhat blue at time of second blood collection. Pancreas normal. |
| | | | 14.5 | 25.8 | 14.4 | 12.42 P. M. | 0.264 | 0.218 | 0.260 | |
| 4 | 5.7 | 24 | 13.5 | 13.7 | 18.15 | 11.50 A. M. | 0.188 | 0.176 | 0.194 | Intestines somewhat blue at second collection. Pancreas normal. |
| | | | 13.9 | 13.7 | 20.7 | 12.30 P. M. | 0.182 | 0.198 | 0.190 | |
| 5 | 11.4 | 24 | 14.7 | 14.1 | 14.9 | 2.59 P. M. | 0.206 | 0.204 | 0.202 | Pancreas and intestines normal. |
| | | | 13.4 | 12.7 | 17.0 | 3.34 P. M. | 0.175 | 0.149 | 0.185 | |

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supply of that substance, or whether there is a decomposition of another character in increased amount, owing to lack of oxygen in the tissues, our experiments do not decide.

In referring to Experiments 4 and 5, the idea has been expressed that the high sugar content found in the control samples was possibly due to the effects of operative disturbance. The question might naturally be raised as to whether the effects noted in our other experiments might not be due to that cause rather than to the influence of adrenalin. To settle this point we have made a series of five control experiments in which the blood was collected in a manner similar to that described, the treatment with adrenalin being omitted. The results are given in Table III, page 47.

These figures show an essential difference from those given in Table II. In only one case does the blood of the hepatic vein contain considerably more sugar than that of the femoral artery. In only one experiment (3) is there an essential rise in the sugar of the femoral blood. The results indicate, therefore, that while in a small percentage of experiments carried out according to this method, the operation may give rise to a hyperglycæmia similar to that produced by adrenalin, in the majority of cases we are justified in attributing the results to the action of adrenalin.

## Sugar in the Blood of the Pancreatico-duodenal Vein after Treatment of the Pancreas with Adrenalin.

It has been shown in the experiments of Series II, page 45, that in the hyperglycæmia which follows the application of adrenalin to the pancreas, the increase of sugar is least in the blood of the portal vein. We have attributed this circumstance to an increased decomposition of sugar in the intestinal tissues, and have suggested that it may be connected with a partial obstruction in the circulation of the blood through those tissues. To ascertain whether the congestion of the pancreas which is regularly observed after treatment of that gland with adrenalin takes part in this phenomenon, we have tested the blood from the pancreatico-duodenal vein. Though an increased decomposition of sugar in the pancreas would be at variance with our ideas regarding the events taking place there, its possibility has not been positively excluded.

The method of collecting blood was as follows: A cannula of the design previously described was introduced into the portal vein at a

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TABLE IV.

SUGAR CONTENT OF BLOOD FROM THE PANCREATIC VEIN BEFORE AND AFTER APPLICATION OF ADRENALIN TO THE PANCREAS.

| No. of experiment. | Wgt. | Time since fed. | Amt. of blood from | | Time of collection. | Time of application of adrenalin. | Time of collection compared with time of application. | Sugar in blood from | | Appearance of pancreas. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Kilo. | Hours. | Femoral artery. Grams. | Pancreatic vein. Grams. | P. M. | P. M. | | Femoral artery. Per cent. | Pancreatic vein. Per cent. | |
| 1 | 13.4 | 22 | | 9.5 | 4.15 | | 13 min. before. | | 0.202 | Normal. |
| | | | | | | 4.27-4.28.05 | | | | |
| | | | | 14.7 | 4.31.05 | | 3 min. after. | | 0.275 | Pale. |
| | | | | 10.4 | 4.37 | | 8 min. 55 sec. after. | | 0.307 | Very slightly congested. |
| 2 | 10.4 | 24 | | 12.4 | 3.57 | | 9 min. before. | | 0.209 | Normal. |
| | | | | | | 4.04.30–4.06.05 | | | | |
| | | | | 10.6 | 4.06.30 | | 25 sec. after. | | 0.277 | Very pale. |
| | | | | 10.1 | 4.11.30 | | 5 min. 25 sec. after. | | 0.287 | Somewhat congested. |
| 3 | 18.6 | 30 | | 12.8 | 4.02 | | 7 min. 20 sec. bef. | | 0.180 | Normal. |
| | | | | | | 4.08–4.09.20 | | | | |
| | | | | 13.2 | 4.10.05 | | 45 sec. after. | | 0.212 | Very pale. |
| | | | | 9.4 | 4.17.40 | | 8 min. 20 sec. after. | | 0.215 | Somewhat congested. |
| | | | | 15.1 | 4.25.35 | | 16 min. 15 sec. after. | | 0.236 | Very much congested. |
| | | | 16.9 | | 4.27 | | 17 min. 40 sec. after. | 0.226 | | |
| | | | | 16.9 | 4.35.35 | | 26 min. 10 sec. after. | | 0.218 | Very much congested. |
| | | | 16.3 | | 4.38 | | 28 min. 40 sec. after. | 0.210 | | |

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point just opposite the entrance of the pancreatico-duodenalis. Loose ligatures were placed about the portal vein, one on either side of the cannula. The cannula was opened at the same time that the ligatures were tightened. Blood is thus obtained from the desired vein, free from admixture with portal blood. The portal circulation is interrupted for a few seconds, but the pancreatic not at all.

We have collected blood in this manner both before and immediately after painting the pancreas with adrenalin, and analyzed it for sugar. The results are given in Table IV.

The results of these experiments are very uniform. In Experiment 1, the percentage of sugar in the pancreatic blood rose 0.073 per cent in the first three minutes after adrenalin was applied. In Experiment 2, an increase of 0.068 per cent occurs within twenty-five seconds. In Experiment 3, the rise in the first forty-five seconds amounts to 0.032 per cent. In the last experiment, we have continued the collection of blood when the gland was very much congested, and have compared these samples with portions taken at the same time from the femoral artery. The analyses show a continued rise in the sugar percentage, and only a slight difference in the blood from the two sources. We are forced to conclude, therefore, that there is not an increase in the decomposition of sugar in the pancreas antecedent to the rise of sugar in the general circulation, and that the difference observed in the second series, between the reducing power of the blood of the portal vein and that of the femoral artery, is not dependent on processes of this nature in that gland.

## Summary of Conclusions.

1. The intraperitoneal injection of adrenalin chloride, as well as the application of that substance to the pancreas, gives rise to a marked increase of sugar in the blood. This hyperglycæmia makes its appearance immediately after the administration, reaches its maximum in from one to three hours, and may continue for over fourteen hours.

2. Simultaneously with hyperglycæmia occurs a decided diminution in the time of extravascular coagulation of the blood. This phenomenon appears to be due also to the application of adrenalin to the pancreas.

3. The cause of this form of hyperglycæmia, as indicated by comparative analysis of the blood flowing to and from the liver, is to be

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attributed, in great part at least, to an increased sugar formation in that organ.

We are indebted to Dr. C. A. Herter for the suggestion of the subject of this work, and for valuable counsel during its progress. We also wish to express our obligation to Mr. William D. Cutter for assistance in a number of the operations.

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# THE IMMEDIATE INFLUENCE OF EXERCISE UPON THE IRRITABILITY OF HUMAN VOLUNTARY MUSCLE.

By THOMAS ANDREW STOREY.

[*From the Physiological Laboratory of Stanford University.*]

IT is commonly the practice of athletes to go through a few preliminary "warming up" movements before attempting a competitive test. Sprinters always take a few short dashes immediately before their events. It is said that horses never make their best records in the first heat of a race.

The writer has noted such facts as these in connection with his work in the Encina Gymnasium of Stanford University, and the following experimental results are presented here because they seem to offer some explanation.

In general, the experimental procedure adopted may be outlined as follows: A single small muscle was substituted for the many groups that participate in normal exercise. The condition of the muscle was made evident by the amount of its contraction in response to the single break-induced current. The muscle was exercised, "warmed up," by voluntary work with an ergograph, and the influence of that work upon the condition of the muscle was then tested by means of the break current.

## Apparatus.

The apparatus employed here has been described by the writer elsewhere. The essential pieces were: an ergograph for the index finger;[1] an electric pendulum for regulating the opening and closing of the primary current to the inductorium, and for cutting out the make-induced current;[2] and an inductorium with 10340 turns on the secondary coil.

In those experiments in which the muscle was excited electrically, the primary current was furnished by three Edison-Lalande cells, "type S," and the secondary coil was placed at a distance of 10 cm.

[1] STOREY: This journal, 1903, viii, p. 355.
[2] STOREY: This journal, 1903, viii, p. 435.



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from the primary coil. The rate of excitation, electrical or voluntary, was once a second. The resistance to contraction in response to electrical excitation was formed by a light, twisted, rubber band which was attached to the recording device. With voluntary excitation the resistance was furnished by spiral springs of different strengths, as indicated with the different figures below.

The first dorsal interosseus muscle of the left hand was used in every experiment.

The writer experimented upon himself in the cases herein reported. Other individuals have been used, and the evidence which they have furnished is the same as that shown below.

## The Influence of a Series of Single Electrical Excitations upon the Irritability of Human Muscle.

It is well known from studies on experimental animals that a muscle stimulated by a succession of induced currents of equal intensity responds by contractions that gradually increase in height; in other words, the irritability of the muscle is increased. Harley[1] has noted this "staircase" in a series of voluntary contractions of human muscle. Waller[2] and the writer[3] have reported it with electrical excitation of human muscle.

Summation caused by electrical stimulation of human muscle has, however, not yet been noted so far as I am aware. I have observed that a strength of the induced current which is at first insufficient to produce a visible contraction of the human abductor indicis will give rise to contraction after from five to fifteen stimuli have been applied. In such a case, subsequent contractions will rise in the staircase form.

## The Influence of a Short Series of Voluntary Contractions upon the Irritability of Human Muscle.

In the two experiments recorded in Fig. 1, the procedure was as follows: The muscle was made to contract a number of times against the resistance of the rubber band mentioned above, in response to

[1] Harley: Journal of physiology, 1894, xvi, p. 100.

[2] Waller: Report to the Scientific Grants Committee, British Medical Association, 1885, p. 68.

[3] Storey: This journal, 1903, viii, p. 373.

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the single break current. After this several seconds were spent in increasing the resistance. In experiment A, a light spiral spring was used, and in experiment B, a heavy spiral spring was added. As soon as the resistance was arranged, the muscle was contracted voluntarily a number of times; but the contractions were not continued long enough to cause fatigue in either experiment. Then followed three or four seconds of rest, during which time the spring resistance was removed. Finally, the muscle was again excited electrically, precisely as in the first part of each experiment.

It is evident that the response of the muscle to electrical excitation with the single induced shock is very much greater after than before

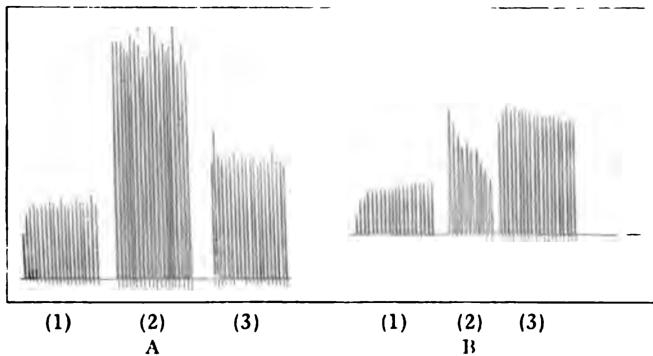


FIGURE 1. — The curves read from left to right. *A* (1), contractions upon electrical stimulation, against very slight resistance; (2), voluntary contractions against considerably increased resistance; (3), conditions as in (1). *B*. Conditions as in Series *A*, except that the resistance to voluntary contraction was very much greater.

a series of voluntary contractions against some resistance. The irritability of the muscle then is considerably increased by a moderate amount of ergographic work, that is to say, by a "warming up" process.

## THE INFLUENCE OF VOLUNTARY MUSCULAR FATIGUE UPON THE IRRITABILITY OF THE WORKING MUSCLE.

In Fig. 2 the experiment is formed of three parts, as in Fig. 1. There is, first, a series of electrical excited contractions, then a series of voluntary, and finally a second series of electrically excited contractions. The voluntary work was done with a moderately strong spiral spring, and was continued until some fatigue was present, as is evident in the curve inscribed.

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There are no staircase contractions in the series of electrically excited contractions beginning the experiment in Fig. 2. This is due to the fact that some muscular work preceded the electrical excitation, so that the period of the staircase contractions had been passed.

On comparing the effect of electrical excitation before fatigue

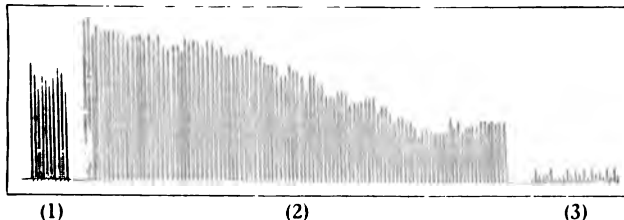


FIGURE 2. — (1) Electrical stimulation, very slight resistance; (2) voluntary contraction against considerably increased resistance; (3) conditions as in (1).

with the effect of electrical excitation after fatigue (Fig. 2), it is evident that the irritability of the muscle has been immediately very greatly reduced by the fatiguing work.[1]

## CONCLUSION.

The experiments herein presented make it evident that human voluntary muscle is made more irritable by successive excitations; that the irritability of human voluntary muscle is immediately very greatly increased by a moderate amount of work; and that it is very greatly decreased by a fatiguing amount of work.

It may be concluded that the "warming up" habits referred to in the opening of this paper are of value in athletic contests, because by such means the irritability of the acting muscle groups is greatly heightened, and they are then able to attain more quickly their maximal strength and speed of contraction.

[1] See also, STOREY: This journal, 1903, viii, p. 369.

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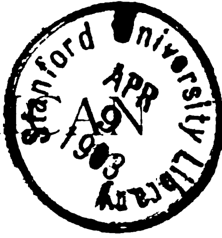
# THE AMERICAN

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# A STUDY OF THE VASOMOTOR NERVES OF THE RABBIT'S EAR CONTAINED IN THE THIRD CERVICAL AND IN THE CERVICAL SYMPATHETIC NERVES.

By S. J. MELTZER and CLARA MELTZER.[1]

[*From the Rockefeller Institute for Medical Research.*]

## Introduction.

SINCE the classical experiments of Claude Bernard and Brown-Séquard on the effects which section or stimulation of the cervical sympathetic nerve exerts upon the blood-vessels of the ear, the sympathetic has been considered to be the carrier of the vasomotor nerves of the ear. In most of the leading text-books no mention is made of the third cervical nerve in this connection. Yet the fact that the auricularis magnus, a branch of the third cervical, carries vasomotor fibres to the ear became known soon after the discovery of vasomotor nerves. As early as 1854, Schiff[2] stated that section of the cervical nerves in which run the fibres of the great auricular nerve causes congestion of the ear, while stimulation of the peripheral end causes constriction of the vessels. Schiff gives no particulars regarding the extent or the intensity of the dilatation. Moreover, according to Schiff, the congestion which follows section passes off in a few hours.

Besides these statements of Schiff, there are in the literature a few reports of other investigations bearing upon this subject. These reports, however, are somewhat contradictory. Lovén[3] (1866) stated that the auricularis magnus contains vasoconstrictors for the tip and the sides of the ear. Pye-Smith[4] (1884) states that in his experiments the great auricular nerve exerts no vasomotor influence upon the vessels of the ear. Morat[5] (1891), who experimented on dogs, concluded that the auricular nerve is a vasoconstrictor of the ear. In one

[1] Research Scholar of the Rockefeller Institute.

[2] Schiff: Beiträge zur Physiologie, 1894, i, p. 148.

[3] Lovén: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1867, p. 1.

[4] Pye-Smith: Journal of Physiology, 1887, viii, p. 25.

[5] Morat: Archives de physiologie, 1892, p. 92.



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experiment in which the right sympathetic was cut eight days before, section of the cervical nerve on both sides caused a congestion of both ears, which was greater on the side on which the sympathetic was intact. Fletcher[1] (1898) studied the effect of stimulation of the peripheral cut end of the third cervical nerve and found that it sometimes causes a constriction even of the proximal third of the central artery. According to Tigerstedt, Moreau[2] also claims that the auricular nerve contains vasoconstrictors for the ear.

Our observations were made on rabbits. We have cut the third cervical nerve on one side and have compared the effect upon the blood-vessels of the various areas of the ear of the same side, with the effect of cutting the cervical sympathetic nerve or of removal of the superior cervical ganglion, either on the other side of the same animal or on the same side of other animals. We have also compared the effect of stimulation of the cut end of the third cervical nerve of one side with that of the stimulation of the sympathetic of the same side, as well as with the stimulation of the third cervical or of the sympathetic nerve on the other side. The effects of cutting the nerves as well as of their stimulation were often studied on one and the same animal. In some cases the effects of stimulation were studied immediately upon cutting the nerves, in other cases the nerves were stimulated many days after section, and in still others the freshly cut nerves were studied on one side, while on the other side the nerves had been cut a few days before.

The hair of the ears was removed by scissors. The use of depilatories, although giving satisfactory results otherwise, was not advisable, on account of their irritating effect upon the skin of the ears, producing a degree of vasodilatation which usually lasted for some time.

## Results of the Cutting Experiments.

The operation was performed while the animal was under ether. When the third cervical nerve was handled, the animal had to be kept under deep anæsthesia. We attached but little importance to the changes which followed immediately upon cutting the nerves while the animal was still under anæsthesia. Frequently both ears are well congested, while the animal is still under the influence of ether, espe-

[1] Fletcher: Journal of physiology, 1897–'98, xxii, p. 259.

[2] Moreau: Memoires de physiologie, Paris, 1872. Quoted from Tigerstedt: Lehrbuch der Physiologie des Kreislaufes, Leipzig, 1893, p. 482.

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cially as long as it remains tied on the board. Indeed the ear on the non-operated side is the one which at times appears the more congested, apparently on account of some actively vasodilating influence of central origin. Immediately upon removal from the board, however, there is already a marked difference in the congestion of the two ears. Our results are derived from many comparisons made at frequent intervals for many days following the operation.

We have made a special study of the subject in hand on twenty-two animals. We have, however, also made a good many more observations on animals which were similarly operated, but chiefly for the purpose of other studies. Our observations brought out the following results:

1. In five out of the twenty-two animals, section of the third cervical nerve was followed by a dilatation of all the blood-vessels of the entire ear, including the central artery. In four of these animals, the sympathetic nerve was cut, or the superior cervical ganglion removed on the other side. The contrast in the congestion of the two ears was striking. The vasodilatation due to the removal of the influence of the sympathetic was confined to the lower two-thirds or three-fourths of the central artery and adjacent parts. The ear on the side where the third cervical was cut was uniformly congested throughout its entire extent and with greater intensity. The central artery was dilated throughout its entire length, and was distinctly wider than on the sympathetic side. In all parts of the ear, numerous fine vessels made their appearance; while the entire ear had a pinkish hue. The picture on page 60 is from a photograph[1] taken ten days after the third cervical nerve was cut on the left, and the sympathetic on the right side. The difference is well represented; but, in reality, the contrast was still more marked.

2. In four animals, section of the third cervical nerve caused only a very moderate dilatation of some blood-vessels at the tip and upper half of the sides of the ear. The subsequent cutting of the branches connecting the third with the second and fourth cervical nerves caused, in one animal, a uniform congestion of the entire ear, in another, a dilatation of the blood-vessels of the entire ear, except the lower two-thirds of the central artery; in the other two animals, the cutting of the connecting branches caused no change.

[1] We are indebted for this photograph to Dr. EDWARD LEAMING, Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.

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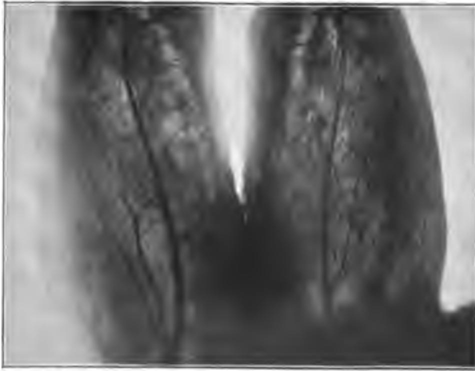
3. The remaining thirteen animals can be put in one group, in which, after cutting the third cervical, all the animals had dilatation of the blood-vessels of at least the sides and top of the ear, including the bifurcation of the central artery, and an absence of dilatation of at least the lower third of the central artery. Within these limits there has been a considerable variation in the extent and in the intensity of the congestion of the ears in different animals. In some, the entire ear looked very congested with the exception of a pale nucleus corresponding to the lower part of the central artery; in others, the ear presented a normal appearance with the exception of a moderate flushing at its periphery. In nearly all cases, the central artery has shown at some point an abrupt transition between the dilated and non-dilated parts. In two cases, the subsequent cutting of the sympathetic filled out exactly the non-dilated part without changing the appearance of the other parts of the ear. In another case, the subsequent cutting of the branches connecting the third with the fourth cervical nerve completed the dilatation, increasing however at the same time the intensity of the congestion of the entire ear. In two other cases, the cutting of the third cervical alone caused originally a congestion of the entire ear; the next day, however, the congestion was found to have become restricted more to the periphery, leaving a pale area at the centre of the ear. For this reason, we put these animals into the third instead of the first group. We have to add that the subsequent cutting of the connecting branches of the third cervical nerve we began only when we were already near the end of our series of experiments. We are therefore unable to state how many of the animals of the third group would have shown a congestion of the entire ear had we subsequently cut these connecting branches.



Rabbit's ears, Experiment 47, ten days after section of sympathetic on the right and cervical on the left side.

Judging from our experiments, it appears that section of the third

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cervical nerve on the left side causes a more extensive congestion than section of the same nerve on the right side. It also appeared that the congestion was greater in gray or brown animals than in white ones. However, the number of animals we have experimented upon is not large enough to permit positive conclusions to be drawn in this direction.

4. The dilatation which followed section of the sympathetic nerve was in the majority of cases restricted to the central artery and the adjacent region. In most of these cases the dilatation of the artery extended only over its lower two-thirds, and in some not even so far. In these cases the dilatation had mostly an abrupt termination. There was one case in which section of the sympathetic caused a congestion of the entire ear; the dilatation of the central artery, however, was not of even width throughout its entire length, but tapered distinctly toward the top. This was in marked contrast with the character of the dilatation of the central artery when it occurred after cutting the third cervical nerve. There was only one other experiment in which the entire ear was congested after section of the sympathetic, but a few hours after operation the congestion had already receded considerably. The difference between the right and left sides which we have noted above with regard to the effects of the section of the third cervical, seemed also to hold good for the sympathetic; the congestion was more marked after section of the left than after section of the right sympathetic nerve. We may add here that in only one experiment did the subsequent removal of the superior cervical ganglion seem to improve somewhat the effect which followed simple section of the sympathetic. In all other experiments the subsequent removal of the ganglion brought no additional changes, not even when the removal of the ganglion occurred a few days after the section of the sympathetic, and the primary effect was already distinctly diminished.

5. Our experiments show that, in general, the sympathetic and cervical nerves carry vasomotor fibres for different areas of the ears, the former controlling the centre, and the latter the periphery of the ear. The influence of the auricularis magnus, however, seems to be, in many respects, greater than that of the sympathetic. (*a*) The congestion of the parts, due to section of the cervical nerves, appeared always more intense than that which followed section of the sympathetic. (*b*) Often the centre was fully congested after section of the cervical nerves, but the periphery was very rarely fully congested after

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section of the sympathetic. (*c*) We noted in two experiments that the central artery, which was dilated after section of the sympathetic, became distinctly more dilated on subsequent cutting of the third cervical nerve. But we find no note in our experiments indicating that the subsequent cutting of the sympathetic improved the congestion of the periphery or of the centre of the ear, which followed section of the third cervical nerve. (*d*) The congestion which follows section of the third cervical nerve (and its connections) persists distinctly longer and in greater intensity than that which follows section of the sympathetic. When the cervical nerve is cut on one side, and the sympathetic on the other, the difference is striking for ten or fourteen days. While the blood-vessels on the sympathetic side become constricted to nearly their original width, and show rhythmical changes again, the ear on the cervical side still looks well congested, with practically no rhythmical changes. In view of our experience we can hardly understand the statement of Schiff that the congestion which follows the cutting of the third cervical lasts only a few hours. On the contrary, in some of the experiments at least, the congestion did not develop fully until some time after the operation.

6. As is well known, and as was mentioned above, the congestion following section of either nerve decreases more or less rapidly. Many writers ascribe this decrease of the vasodilatation to the assumption of the constricting tonus by some uncut vasoconstrictor nerve fibres, which normally do not participate in the maintenance of the tonus, but which, in the absence of the chief constrictors, are ready to substitute the latter, — a sort of *collateral innervation*. In our experiments, we had a few animals in which on one side the superior cervical ganglion was removed, and the third cervical nerve and all its connecting branches were cut. We had reason to believe that we had thus excluded all vasoconstrictors of the ear. Nevertheless, a few weeks after the operation, there was little left of the original marked congestion. In these cases, the recurrent constriction could not be the work of substitution or collateral innervation. Neither could it be the result of regeneration. Aside from the shortness of time, the occasional *autopsies in vivo* have shown that there was as yet no regeneration. Apparently the walls of the blood-vessels themselves possess the capacity of resuming their tonicity without the aid of extrinsic nerve influence. Furthermore, we had experiments which seem to demonstrate that at least in these cases no substitution took place. For instance, the ganglion was removed and a dilatation of the

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central artery followed. When, later, after this dilatation had disappeared, the third cervical and its connecting branches were then cut, a congestion of the periphery appeared while the centre remained pale. In this case the subsequent constriction of the central artery was apparently the work of the blood-vessel itself, and was not due to an assumption of the tonus by the other vasoconstrictor fibres of the ear. It is true, we had a number of experiments in which, after the congestion following the primary cutting of the sympathetic had diminished, a subsequent cutting of the cervical nerves caused, in addition to the congestion of the peripheral parts of the ear, also a redilatation of the central artery. This, however, does not necessarily mean that the cervical assumed the control over the central artery only after the sympathetic had been cut, since we know, as we have seen above, that the primary cutting of the cervical nerves alone often causes a dilatation of the central artery.

Our experiments, therefore, furnish evidence that the constriction of the blood-vessels can return apparently without the aid of extrinsic nerves. There is, furthermore, satisfactory evidence that, at least in some cases, when the cervical nerve does not participate originally in the maintenance of the tonus of the central artery, this nerve does not assume the tonus after the influence of the sympathetic is eliminated. Finally our experiments afford no sufficient evidence that a collateral innervation ever takes place in the tonus of the vasomotor nerves of the ears; *i. e.*, we have no evidence that the cervical can take the function of the sympathetic, or the sympathetic that of the cervical nerve, if the substituting nerve had originally no active share in the maintenance of the vascular tonus.

On the other hand, we had experiments in which the dilatation of the central artery due to the cutting of the sympathetic was distinctly increased throughout the entire length of the artery by cutting the cervical nerve a few minutes later. This can only mean that the vasomotor tonus of the entire central artery was simultaneously maintained by the sympathetic as well as by the cervical nerves. This condition was observed to exist more frequently in the tonus of the upper part of the artery. Our observations in this respect, however, are too few in number to permit a detailed discussion of these complicated conditions.

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## Results of Stimulation Experiments.

As far as we know, Fletcher is the only investigator who has given a detailed account of the effect of stimulation of the third cervical nerve. According to his experience, electrical stimulation causes the greatest constriction in the distal third of the central artery, but the proximal third also shows a marked constriction. To obtain an effect from this nerve, Fletcher states that the stimulating current must be stronger than that which elicits an effect from the sympathetic. Again, the latent period in the stimulations of the third cervical nerve is much longer than that in stimulations of the sympathetic. Finally, according to Fletcher, there is a difference in the order of the return of the flush after discontinuation of stimulation. After stimulation of the sympathetic, the return flush begins distally and travels towards the base of the ear; after stimulation of the third cervical nerve, it begins proximally and affects the terminal bifurcation of the artery last.

Our stimulation experiments were made on sixteen animals. In many of them the cervical nerve as well as the sympathetic was stimulated on both sides. In a few cases, the nerves were stimulated five days after they had been cut. We shall give only a brief summary of our results.

7. For the majority of animals, it can be stated in a general way that stimulation of the third cervical nerves caused chiefly a constriction of the vessels of the sides and top of the ear, including also the upper end of the central artery; stimulation of the sympathetic caused chiefly a constriction of the lower three-fourths of the central artery and adjacent parts of the ear. In a smaller number of cases, stimulation of the third cervical nerve caused also a complete constriction of the entire central artery, and in very few instances did stimulation of the sympathetic cause pallor also in the sides and top of the ear.

8. In nearly all cases, the pallor following stimulation of the third cervical nerve spread from the top downward, and the constriction of the artery caused by stimulation of the sympathetic spread from the base of the ear upward. Regarding the order of the refilling of the vessels after discontinuation of the stimulation, we have too few notes to permit a general conclusion to be drawn.

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9. Regarding the intensity of the constriction which is caused by the stimulation of either nerve, we found in our experiments no difference in favor of the sympathetic. On the contrary, in our experiments the general pallor, as well as the degree of constriction of the individual vessels, was often greater after stimulation of the cervical nerve than after that of the sympathetic. The same must be said of the strength of the stimulus which is required to bring out an effect from each nerve, and of the length of the latent period in each case. Fletcher as well as Morat state that the interrupted current which is sufficient to cause constriction upon stimulation of the cervical nerve, must be considerably stronger than ordinarily employed in nerve stimulation. Fletcher ascribes this to the thickness of the sheath of the cervical nerves. In our experiments, we had a relatively large number of instances in which a weaker current brought out a strong effect from the cervical nerve, while a stronger current brought out a comparatively slight effect from the sympathetic of the same side. For instance, in two experiments, stimulation of the right cervical nerve with the interrupted current, while the secondary coil was at a distance of 130 mm., caused great pallor, and a constriction of the blood-vessels of the entire ear, except the lower two-thirds of the central artery; while stimulation of the right sympathetic with the secondary coil at a distance of only 90 mm., caused only a very moderate constriction of the lower two-thirds of this artery. Our experience with regard to the strength of the stimulus is as follows: There have been cases in which equally strong currents brought out equally strong effects from both nerves. In other cases, as stated above, a weaker current brought out a strong effect from the cervical nerve, and a stronger current caused a mild effect from the sympathetic. In still other cases, only a strong current elicited some effect from the cervical nerve, while a weaker current elicited from the sympathetic a constriction leading to the entire disappearance of the central artery. Only this latter class of cases, but few in number, corresponds with those seen by Fletcher.

Similar variations were observed with regard to the latent period. But here we can say, in general, that in all cases in which the stimulation caused a strong effect, the latent period was short. When the effect was moderate, and the current had to be strong, the effect set in later. It would seem to us that the thickness of the sheaths of the nerves has little to do with these variations; they seem to be due rather to individual variation, which can, of course, only be recognized

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when the experiments are extended over a large number of animals. Fletcher seems to have experimented upon only four animals.

We have also notes on the length of the after-effect in some experiments. As far as the few data permit any conclusion, it would seem that the long after-effect followed generally moderate primary effects brought on by strong currents. When the primary effect was a strong one, the return flush usually set in very soon after the stimulation was discontinued. However this may be, we can positively state that the long and short after-effects were equally divided between the cervical and the sympathetic nerves.

We have thought it necessary to dwell especially upon these points, because the strength of stimulus, latent period, and after-effect are criteria sometimes employed to distinguish between different kinds of nerves, and might have been looked upon also in our case as physiological criteria distinguishing the sympathetic from the cervical nerves. Furthermore, such a physiological distinction would seem to run parallel with a certain anatomical distinction which appears to exist between the sympathetic and the cervical nerve fibres. According to the investigations of Fletcher, the fibres which pass to the ear by the route of the cervical nerves arise from the cells of the ganglion stellatum. These vasomotor fibres are therefore post-ganglionic, while the vasomotor fibres within the cervical sympathetic are preganglionic. We could then be misled into the belief that the longer latency of period, and the requirement of greater intensity of stimulus, etc., might be qualities peculiar to post-ganglionic nerve fibres. We therefore took occasion to state especially that in our experiments these qualities were not peculiar to either set of nerve fibres, but seem to depend rather upon the individual variations of the animals. The basis of these variations, whether they are simply due to variations in the distribution of the number of vasomotor fibres of the same character between the cervical and sympathetic nerves, or whether there is a variation in the distribution of fibres of different character, we do not wish to discuss for the present.

10. In nearly all cases in which both sides were compared in the same animal, stimulation of the left sympathetic gave a distinctly better effect than stimulation of the right, with regard to the degree as well as the extent of the constriction. This holds good in a general way also for stimulation of the cervical nerves; here, however, we had one animal in which stimulation of the right cervical nerve gave the better effect, and two in which the effect was about equal on both sides.

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This effect of stimulation coincides with the above-mentioned effect of section of the nerves in which the congestion was found to be often more intense on the left than on the right side. While the number of our experiments is, perhaps, as yet too small to justify the general conclusion that in all rabbits the left nerves contain a larger number or more efficient vasomotor fibres than those on the right side, it is at least well to keep in mind that this was the case in a number of consecutive experiments. The importance of this will become evident when we consider that among the methods employed to study the question, whether there is any difference in the effect between simple section of the cervical sympathetic, and the removal of the superior cervical ganglion, there was one which consists in the comparison of the effects following section of the sympathetic of one side and removal of the ganglion on the other side. In view of our experience, it is obvious that this method is incapable of leading to decisive results. This method presupposes that the effect of section of the sympathetic, or the removal of the ganglion, is in all animals the same on both sides. Now assuming even that the greater effect obtained on the left side in our experiments was a matter of accident, these experiments demonstrate unmistakably that presupposition of similarity on both sides is not permissible, and therefore any inference drawn from a method which contains this supposition as a premise cannot be conclusive.

11. In our experiments, we met with cases in which the stimulation of the nerves had but little effect, while their section brought out a considerable flushing of the ear. This shows that there is a difference between the normal stimulation which maintains the arterial tonus, and the artificial stimulation of the end of the cut nerve. This difference might be due to the fact that the normal tonus is maintained by stimuli which are certainly more adequate for nerve excitation than the electrical current; but it might also be explained by the assumption that the normal stimulus affects solely or preferably one set of nerve fibres, while the artificial stimulus affects simultaneously and indiscriminately two antagonistic sets of fibres. This recalls the relations between the inhibitory fibres of the vagus and the accelerating nerve fibres. While a simultaneous artificial stimulation of both kinds of nerve fibres always favors inhibition, nevertheless reflex acceleration is possible, and it seems that a normal tonus of the accelerating nerve fibres is being continually maintained.

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## Summary.

The more essential points of our investigation are:

1. In the majority of cases, the third cervical nerve carries the vasomotor fibres for the blood-vessels of the rabbit's entire ear, except for a comparatively small area around the lower two-thirds of the central artery, for which the sympathetic is the carrier of the vasomotor fibres. In a good many cases, the cervical nerve innervates also the vessels of the centre, and in very few exceptional cases, the sympathetic carries fibres also for the periphery. There is probably in all cases a zone in which the vascular tonus is maintained by the nerve fibres of both nerves simultaneously.

2. The congestion following the section of the cervical nerve lasted in all cases longer than that following the section of the sympathetic.

3. Section as well as stimulation of both nerves caused a distinctly better effect on the left side than on the right.

4. There was no constant proportion between the effect of section and that of stimulation.

5. Even after section of all the vasomotor carrying nerves, the blood-vessels, sooner or later, become constricted again, probably through some intrinsic activity of the blood-vessels themselves.

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# THE SPECIFIC ROTATION OF THE NUCLEIC ACID OF THE WHEAT EMBRYO.

By THOMAS B. OSBORNE.

[*From the Laboratory of the Connecticut Agricultural Experimental Station.*]

GAMGEE and Jones[1] have recently described nucleoproteids from several sources, all of which showed the unexpected property of right polarization. As it is possible that the nucleic acid component may, in whole or in part, be the cause of this dextro-rotation, I have, following the suggestion made by Dr. Gamgee, examined the rotation of the nucleic acid which I have obtained from the wheat embryo.[2]

The specific rotation was determined by suspending the dry acid in water and gradually adding decinormal potassium hydroxide solution until all was dissolved. In this way a perfectly clear solution was obtained which reacted strongly acid with litmus and contained the nucleic acid as acid potassium nucleate. The rotation of the solutions was observed at 20°, with the following results: —

1. Observed angle +3.16°. Amount of dissolved acid =.0236 gr. per c.c. Length of the tube 2 dm: —

$$(\alpha)_D^{20^\circ} = +66.95^\circ.$$

2. Observed angle +5.84°. Amount of dissolved acid =.0400 gr. per c.c. Length of tube 2 dm: —

$$(\alpha)_D^{20^\circ} = +73^\circ.$$

3. Observed angle 2.89°. Amount of dissolved acid =.0393 gr. per c.c. Length of tube 1 dm: —

$$(\alpha)_D^{20^\circ} = +73.53^\circ.$$

[1] GAMGEE and JONES: This journal, 1903, viii, p. 447.

[2] OSBORNE and HARRIS: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 85; also Report Connecticut Agricultural Experimental Station for 1901.



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These solutions showed no change in rotation after standing twenty-four hours.

From these results it is evident that this nucleic acid is strongly dextrorotatory and that the degree of rotation is considerably influenced by the concentration of the solution.

In order to determine the rotation of a mixture of protein substance with nucleic acid, a quantity of triticonucleic acid was dissolved in water with addition of an amount of potassium hydroxide which just sufficed for solution, and then one half as much pure ovalbumin was added, and the solution examined in a 200 mm. tube, with the following result: —

4. Observed angle 2.50°. Amount of dissolved substance .0404 gr. per c.c. Length of tube 2 dm: —

$$(a)_D^{20°} = +30.94°.$$

This is approximately the rotation calculated for a mixture of one part of ovalbumin $(a)_D^{20°} = -30°$ and two parts of wheat nucleic acid $(a)_D^{20°} = +67°$, the mean rotation of which would be $+35°$.

It is thus evident that a combination of protein substance with nucleic acid may show strong right polarization, and that this dextrorotation may be wholly due to the nucleic acid component.

Gamgee and Jones give no data from which the proportion of protein and nucleic acid in their nucleoproteids can be inferred, except in the case of "Hammarsten's preparation," which designation, although they do not say so, presumably refers to the nucleoproteid obtained by Hammarsten's method from the pancreas.[1] This nucleoproteid according to Hammarsten, contains 4.5 per cent of phosphorus, from which we may assume that it contains about 50 per cent of nucleic acid, since Levene[2] found 8.65–9 per cent of phosphorus in his preparations of the nucleic acid of the pancreas. If the dextrorotation of the nucleoproteid of the pancreas is wholly due to the nucleic acid, the specific rotation of the acid in "Hammarsten's preparation" must be very high in the light of Gamgee's and Jones's figure. This, however, was obtained in such an extremely dilute solution that it is quite possible that it is too high.

The figures given for the other nucleoproteids are such as might be caused by the dextrorotation of the nucleic acid. That this is the

[1] Hammarsten: Zeitschrift für physiologische Chemie, 1894, xix, p. 19.
[2] Levene: *Ibid.*, 1901, xxxii, p. 548.

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case is indicated by the fact that, as Gamgee and Jones say, "The specific rotation of the nucleoproteid is +38°, that of the nuclein +65°, while it can be indirectly shown that a substance is contained in the residual material whose specific rotation is about +81°."

We thus see that with a probable increase in the proportion of nucleic acid in the compounds examined there was an increase in the dextrorotation.

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# THE INFLUENCE OF COLD ON THE ACTION OF SOME HÆMOLYTIC AGENTS.[1]

By G. N. STEWART.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

I HAVE already shown[2] that sapotoxin causes an increase in the electrical conductivity and in the permeability to electrolytes of formaldehyde-hardened red corpuscles, and have, without being able to directly prove this, brought forward evidence that it exerts a similar action on the unfixed corpuscles. The difficulty in making observations on the latter consists in the rapidity with which sapotoxin causes laking. This increases the conductivity by the liberation of electrolytes on the one hand and diminishes it by the liberation of hæmoglobin on the other, and so obscures the phenomenon which comes so clearly to light in the case of formaldehyde corpuscles. The fact that at 0° C. certain of the biological laking agents are said not to act, suggested that by cooling the blood to 0°, and using small doses of sapotoxin, an action on the conductivity might be revealed before any laking occurred. Dr. Peskind showed that, as a matter of fact, under the conditions mentioned, laking is delayed for a considerable period, a period which, as I have found, may extend to many hours.

The procedure was as follows. The dose of sapotoxin necessary to cause laking of blood at air temperature was first determined. Then a measured volume of defibrinated blood was cooled in ice to 0°, and a predetermined quantity of ice-cold sapotoxin solution (of course in NaCl solution) added to it. The mixture was rapidly shaken up and returned to the ice. A control specimen of defibrinated blood containing as much of the 0.9 per cent NaCl solution used in making the sapotoxin solution,

[1] Some of the experiments included in this paper were made in conjunction with Dr. S. PESKIND, in the Physiological Laboratory, Western Reserve University, during his tenure of the H. M. Hanna Fellowship.

[2] Journal of physiology, 1899, xxiv, p. 211; 1901, xxvi, p. 470; Journal of experimental medicine, 1902, vi, p. 257.



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as was added to the blood of the sapotoxin solution, was also kept in the ice. Another specimen of defibrinated blood, containing as much of the sapotoxin solution as the first, was kept at air temperature. From time to time resistance measurements of these mixtures were made, a measuring tube being immersed in crushed ice, into which dipped a thermometer. The readings of the thermometer never varied more than one or two tenths of a degree from 0°. The tube containing the electrodes was always immersed for some time in the ice before the blood mixtures were poured into it. In the tables the conductivities (at 0°C.) are expressed in reciprocal ohms $\times 10^8$. The commencement and progress of laking in the mixtures was controlled by putting from time to time into a cooled graduated centrifuge tube 1 c.c. of the mixture, filling up the tube to 15 c.c. with the ice-cold salt solution, and centrifugalizing in a cold room with a centrifuge of such speed that complete sedimentation occurred in four and one-half minutes. The amount of hæmoglobin, if any, in the supernatant liquid was then determined colorimetrically.

In this way it has been shown that sapotoxin, when added to defibrinated blood at 0°, produces for some time no effect on the conductivity and no laking. For example, in Experiment I, a dose of 2 per cent sapotoxin solution, corresponding to 6 c.c. to 100 c.c. of blood, caused for at least two and one-half hours no change in the conductivity and no laking. Then an increase in the conductivity developed, which reached its maximum after about twenty-one hours, practically no laking having taken place up to this time. The dose of sapotoxin was insufficient to cause any increase in the conductivity of the specimen left at ordinary temperature, except for the relatively short period before laking had begun, or while the amount of hæmoglobin liberated was still insignificant.

In Experiment II, with a different specimen of blood, but the same dose of sapotoxin, the period of delay before a change of conductivity occurred was much less. In one hour, an increase in conductivity was marked, and indeed had reached its maximum; whereas in thirteen minutes it had not begun. In one hour and thirteen minutes, the blood had not begun to lake. This dose was sufficient to cause a slight increase in the conductivity of the blood laked at ordinary temperature.

In Experiment III (1), in which the blood was practically fresh, the same dose of sapotoxin caused but little increase of conductivity for an hour. After two hours, the conductivity was distinctly, and after eighteen and one-half hours, markedly increased, while no laking

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took place before the twenty-third hour. After forty-eight and one-half hours only 15 per cent of the hæmoglobin was in solution. The dose of sapotoxin was sufficient to cause an increase of conductivity in the partially laked blood left at room temperature, but when laking was almost complete this increase was completely masked by the liberated hæmoglobin. When, to the same defibrinated blood after standing in the cold for twenty-four hours, a dose of sapotoxin two-thirds greater was added, a distinct increase in the conductivity had already taken place in thirteen minutes. The maximum conductivity (before laking) occurred in thirty minutes, and obvious laking had appeared within one and one-half hours of mixture. Notwithstanding the depressing influence of the liberated hæmoglobin, the conductivity was increased by laking (both in the specimen kept at 0° and in that kept at air temperature), as always happens on the addition of a dose of sapotoxin more than sufficient to just cause laking, owing to the liberation of electrolytes from the corpuscles.

In Experiment III (2), the effect of an increased dose of sapotoxin (equal to 10 c.c. of the 2 per cent solution to 100 c.c. of blood) was tried on a specimen of the same blood as was used in Experiment III (1). While there is some increase of conductivity before laking, the typical effect of the large dose is seen in the marked increase of conductivity after laking, particularly in the mixture kept at air temperature.

In Experiment IV, with perfectly fresh blood, and a dose of sapotoxin corresponding to 8 c.c. of the solution to 100 c.c. of blood, the maximum change in conductivity was produced in thirty-two to thirty-eight minutes, before which laking had not begun.

In Experiment V, with the same blood as in Experiment IV, but after keeping it forty-eight hours in ice, a dose of 6 c.c. of sapotoxin solution to 100 c.c. of blood, produced a slight increase of conductivity, even in five minutes after mixture, and the change went on increasing till a maximum (without laking) was reached in about one and one-fourth hours. For one hour and eight minutes longer, no laking occurred, the conductivity maintaining itself at the level previously reached. The dose of sapotoxin was sufficient to cause a distinct increase in the conductivity of the blood allowed to lake at air temperature, in other words, it was sufficient to cause a liberation of electrolytes which, in spite of the discharged hæmoglobin, increased the conductivity.

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The simplest explanation of these phenomena is that the sapotoxin, when it acts at 0°, produces first such an effect on the superficial layer (envelope) of the corpuscles, that its permeability to electrolytes is increased. This change does not necessarily, or at any rate does not immediately, lead to a discharge of hæmoglobin. The second stage of the action of the sapotoxin consists in the liberation of the hæmoglobin, presumably by breaking up the compound which it forms with the stroma. This obviously will be a gradual process, since the penetration of the sapotoxin will take time, especially when it is delayed by a low temperature. If the dose of sapotoxin is minimal, it does not appear that the process goes beyond this second stage, either at 0° or at air temperature. But if the dose is greater than that just sufficient to cause the change in the envelope and the discharge of the hæmoglobin (with, it may be, such portion of electrolytes as exists in ordinary solution in the corpuscles), the electrolytes which are bound to the stroma, chemically or physically, appear to be liberated, and the conductivity of the laked blood is increased more or less markedly in proportion to the dose. The decided increase which is produced in the potential difference between the belly and the tendinous extremity of the frog's gastrocnemius, when sapotoxin solution (in 0.9 per cent NaCl) is applied to the muscle next the tendon, may be due either to a change in the permeability of the sarcolemma, or to the liberation of electrolytes in the fibre contents, or to both. A similar, though less marked, increase is produced by sapotoxin in the current of rest of nerve.

It may be asked whether there is any proof that the sapotoxin is taken up and fixed by the corpuscles during the stage when the conductivity is increasing, without any laking having been produced. Clear evidence has been obtained that this is the case.

For example (as in Experiment I), measured quantities of the mixture of blood and sapotoxin kept at 0° were removed after the increase of conductivity had clearly developed itself, but before any laking had occurred. They were rapidly centrifugalized in a cold room after being shaken up with a known volume of ice-cold salt solution (usually 14 times as much as was taken of the blood). The supernatant liquid, seen to be free from hæmoglobin, was decanted off, and the washing with ice-cold salt solution and centrifugalizing repeated once or twice more. In this way, all the sapotoxin not in the corpuscles was removed. The sediment was then distributed in salt solution, and left at air temperature or placed in a bath at 40°C., and it was seen that laking occurred. The amount of

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laking was determined by centrifugalizing and estimating colorimetrically the quantity of hæmoglobin in solution.

It was found that the ultimate amount of laking was not markedly less in the specimens of sapotoxin blood kept at 0° and washed free from extraneous sapotoxin than in similar specimens of sapotoxin blood kept at air temperature, and then washed free from extraneous sapotoxin. Not only then do the corpuscles fix sapotoxin at 0°, but, apparently, at the stage when the conductivity is increased while no actual laking has yet taken place, they have fixed as much, or nearly as much, as they would have done had laking been allowed to proceed at air temperature. Formaldehyde-hardened corpuscles also fix sapotoxin, as can be shown by adding to a suspension of the washed corpuscles in salt solution a small amount of sapotoxin, allowing the mixture to stand and then centrifugalizing. The supernatant liquid will be found to have no hæmolytic power.

Since, as is well known, the cholesterin of the serum neutralizes the action of a certain amount of sapotoxin and to this extent prevents it from laking the corpuscles,[1] it was thought that still more striking effects on the conductivity of the corpuscles would be obtained if a suspension of corpuscles washed free from serum constituents were employed. Experiments VI and VII were performed in order to test this, but to my surprise, I could obtain no distinct evidence that the phenomenon in question is to be observed with washed corpuscles at all. The observations are rendered more difficult by the great sensitiveness of washed corpuscles to sapotoxin. Thus in Experiment VI a suspension containing about 75 per cent of corpuscles (by volume) was laked almost immediately at 0° by addition of sapotoxin in the proportion of 2 c.c. of the 2 per cent solution to 100 c.c. of the suspension, and so violent was the action, that no intact red corpuscles could be discovered by the microscope, although swollen leucocytes were plentiful. In accordance with the size of the dose the conductivity of the laked blood was greatly increased. Partial laking occurred very rapidly when sapotoxin was added in the proportion of 1.4 c.c. of the 2 per cent solution to 100 c.c. of the suspension. Even the addition of sapotoxin in an amount corresponding to 0.6 c.c. of the 2 per cent solution to 100 c.c. of the suspension caused

[1] Cholesterin suspended in saline solution removes sapotoxin completely from solution, and prevents the laking of blood added to the mixture. After filtering off the cholesterin, the filtrate has no laking action.

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such a rapid laking that the decline of conductivity associated with the escape of the hæmoglobin was proceeding three to five minutes after mixture. That the dose was by no means a large one (as tested by its effect in liberating electrolytes from the corpuscles) was shown by the fact that, instead of any increase in the conductivity being produced as laking went on, a marked and progressive diminution occurred. It was shown by the method already described that the corpuscles had fixed some sapotoxin, which produced a further laking after all extraneous sapotoxin was removed.

In Experiment VII, in which a minimal dose was much more carefully sought for than in Experiment VI, the attempt to discover an increase of conductivity preceding the liberation of hæmoglobin from washed corpuscles was unsuccessful, although the corpuscles had fixed enough sapotoxin to cause a considerable amount of further laking. It could not be determined whether any sapotoxin was fixed before laking began, since the interval between addition of the sapotoxin and the commencement of laking was so short even at 0°. On the other hand, it was quite clearly shown that in the case of the washed corpuscles, as well as of the unwashed, the laking process is much retarded by the low temperature.

If in reality the preliminary stage of increased conductivity without liberation of the hæmoglobin is not passed through in the laking of washed corpuscles, two explanations suggest themselves: (1) that the preliminary increase of permeability of the corpuscles in entire blood is produced not by the sapotoxin itself but by a compound of the sapotoxin with some constituent of the serum, the cholesterin, *e. g.*, which compound, less violent in its action than the sapotoxin, may affect the envelope of the corpuscles without liberating the hæmoglobin; (2) that the ions to which the corpuscles become more permeable do not include Na and Cl. The former would appear the more probable hypothesis and I hope that further experiments will settle the point. A possible diminution in the internal friction produced by some action of the sapotoxin on the serum, which, of course, would cause an increase in the velocity of the ions and therefore an increase in the conductivity, is excluded by the fact previously shown[1] that the addition of sapotoxin to serum does not increase its conductivity. Further, the gradual increase in conductivity of the sapotoxin blood at 0° is against this idea.

[1] STEWART: Journal of physiology, 1901, xxvi, p. 484.

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This experiment shows well, as several of the others do, that with minimal doses of sapotoxin there is for a long time, even when laking is allowed to go on at ordinary temperature, little or no liberation of electrolytes from the corpuscles. Instead of an increase of conductivity there is a diminution due to the depressing influence of the discharged hæmoglobin, and approximately proportional to the amount of blood-pigment set free. Thus in A′ (Experiment VII) when 57 per cent of the total hæmoglobin was in solution λ was 40.50, while for the "control" λ was 47.92. If we take the proportion of hæmoglobin in the corpuscles as 40 per cent, and the proportion of corpuscles in the suspension (calculated from the conductivity), as 36 per cent, the hæmoglobin in A′, if it were all liberated, would make up a solution containing 13.8 per cent of blood pigment. With 57 per cent of the hæmoglobin discharged into a volume of "serum" constituting 64 per cent of the suspension and about 65.4 per cent of A′, the serum would contain about 12.2 per cent of hæmoglobin. But the serum is, of course, increased by the escape of water from the laked corpuscles. On the assumption that 57 per cent of the corpuscles has been completely laked (an assumption not perfectly correct, since partial laking of some corpuscles must have taken place), the amount of serum would be about 77 per cent, and the proportion of hæmoglobin dissolved in the serum about 10.3 per cent. I have shown [1] that when one gram of oxyhæmoglobin is dissolved in 100 c.c. of serum, the conductivity is depressed by 1.82 per cent. If the conductivity of the control (in Experiment VII) be diminished by 1.8 × 10.3, that is, 18.5 per cent, we get 39.1, which is not very different from the actually observed λ of A′ (40.5). The liberation of a small amount of electrolytes from the laked corpuscles and the increased permeability of the unlaked corpuscles would easily account for the slight difference.

The addition of a further amount of sapotoxin to A′ caused a very great increase in λ. In previous researches I did not study the effect of really minimal doses of sapotoxin. My former conclusion, that sapotoxin causes the liberation of electrolytes in considerable amount from the corpuscles during laking, must therefore be supplemented by the statement that this is the case only when doses greater than those just sufficient to produce laking are employed. After a minimal dose of sapotoxin has caused the liberation of the hæmoglobin from the corpuscles, unaccompanied by any large proportion of the electrolytes of the latter, the addition of a further quantity of the poison brings about a marked discharge of electrolytes from the stromata, just as the addition of water or sapotoxin to the ghosts of heat-laked blood causes the liberation of electrolytes.

[1] STEWART: Journal of physiology, 1899, xxiv, p. 216.

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Since, as previously shown, the conductivity of formaldehyde-fixed corpuscles is increased by sapotoxin at air temperature, it became of interest to determine whether cooling to 0° abolishes this effect. Experiment VIII shows that the increase of conductivity is practically as great as at air temperature. Apparently a low temperature does not much, if at all, retard the action, at least for the dose employed.

This result is suggestive as regards the portion of the formaldehyde-fixed corpuscle affected by the sapotoxin. If the sapotoxin had to penetrate to the interior of the corpuscle in order to produce the change of conductivity, the low temperature might have been expected to markedly retard the effect. The fact that the retardation is not striking corroborates the view taken in previous papers that it is the surface layer (envelope) of the formaldehyde corpuscle which is attacked by it. I take the opportunity of mentioning here certain results obtained by Dr. C. C. Guthrie in my laboratory in an investigation, which is still proceeding, on the influence of formaldehyde in various doses on blood, and especially on the action of laking agents. He finds that if blood (dog's or rabbit's) be drawn into an equal volume of 1 per cent formaldehyde solution in 0.9 per cent NaCl solution, the corpuscles can be laked (by water or sapotoxin) after many hours. Even after forty-eight hours, a mixture of one part of blood with two parts of 1 per cent formaldehyde solution (which, like mixtures containing still larger amounts of formaldehyde, remained unclotted) was laked by water and more slowly and partially by sapotoxin. In most specimens of dog's blood, clotting is completely prevented when the formaldehyde present amounts to one volume of the 1 per cent solution to 0.6 volume of blood. In some specimens somewhat more is needed, in others less. Much smaller quantities of formaldehyde (*e.g.*, two to twenty parts of blood to one part of 1 per cent formaldehyde) retard coagulation in proportion to the quantity of formaldehyde present, and although they do not prevent its ultimate appearance, they exert a marked influence on the process, as is shown by the slow and imperfect shrinking of the clot as compared with that in control specimens of normal blood. One of the ways in which formaldehyde prevents or retards coagulation seems to be by hindering the development of the fibrin ferment (perhaps by a rapid partial fixing of the leucocytes or blood-plates), and this appears to be a more powerful action than its inhibition of fibrin ferment already formed. The hæmolytic action of dog's serum on rabbit's corpuscles

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is hindered by formaldehyde in proportion to its concentration and the time it has acted. This is true whether the formaldehyde is added to the dog's serum or to the rabbit's blood, and indeed the addition of a given amount to the dog's serum causes a greater diminution in the hæmolytic activity than the addition of the same amount to the rabbit's blood, when the formaldehyde is allowed to act for the same period before mixing the blood and foreign serum. The laking of the blood by such hæmolytic agents as have been investigated is not essentially altered, even after a considerable period, by such quantities of formaldehyde as are necessary to prevent putrefaction.

It might be expected that other hæmolytic agents than sapotoxin would produce a similar effect on the permeability of corpuscles as a preliminary to laking. I investigated bile salts (Na taurocholate) as a representative of the other chemical lakers, and foreign serum as a representative of the biological lakers. As is shown in Experiment IX, while there appeared to be a slight increase of conductivity produced by the bile salt in the case of unwashed corpuscles, it is not so pronounced as when sapotoxin is added. In this connection it may be recalled that bile salts, while they increase the conductivity of formaldehyde-fixed corpuscles, do not cause so great an increase as sapotoxin.[1] A further fact, presumably related to this difference, is that in sapotoxin laking the corpuscles always swell before discharge of the hæmoglobin, becoming smooth in outline if they have previously been crenated, and therefore take up water, while in sodium taurocholate laking they need not do so. In Experiment X, an attempt was made to determine whether the conductivity in a suspension of washed corpuscles was increased by sodium taurocholate before laking, but with the same negative result as in the case of sapotoxin. The sensitiveness of the corpuscles to the bile salt is increased by the removal of the serum constituents just as in the case of sapotoxin.

The fact that some of the biological hæmolytic agents, *e. g.*, snake venom, according to Flexner and Noguchi[2] are unable to cause laking in washed corpuscles, while in the absence of serum constituents sapotoxin and bile salts are apparently unable to produce the preliminary increase of permeability of the corpuscles, might seem to suggest that such biological lakers may act primarily by augmenting the permeability of the envelope; and that just as in the absence

[1] STEWART: Journal of medical research, viii, p. 268.

[2] FLEXNER and NOGUCHI: Journal of experimental medicine, 1902, vi, p. 286.

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of the complement of the serum, the venom is unable to complete the reaction on which this increase of permeability depends, so, in the case of these chemical lakers, something which can act as a complement or as an intermediary body, to use Ehrlich's terminology, is necessary to produce the preliminary increase of conductivity, although ultimately laking can take place without it. In Experiment XI, however, no evidence was obtained that dog's serum can produce any change in the conductivity of rabbit's blood before liberation of the hæmoglobin. This is in agreement with the fact that the permeability of the formaldehyde-fixed corpuscles of the rabbit is not affected by dog's serum. It was observed that with foreign serum complete laking will take place at 0°, although the statement has been made that it does not occur below about 5° C.

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EXPERI-

February 8.—At 12.20 P.M. added to 25 c.c. of dog's defibrinated blood obtained nineteen hours before and kept in cold 1.5 c.c. of 2 per cent sapotoxin solution in 0.9 per cent NaCl solution, both ice-cold. Call the mixture A. Kept in ice. At 11.55 A.M. added to 25 c.c. of the defibrinated blood 1.5 c.c. of 0.9 per cent NaCl solution. Call the

| P. M. | | λ[1] |
|---|---|---|
| 12.38 | Control cooled in ice before putting in U tube . . . . . . | { 34.73 |
| 12.40½ | . . . . . . . . . . . . . . . . . . . . . . . . | 34.63 |
| 12.25 | A . . . . . . . . . . . . . . . . . . . . . . . | { 34.96 |
| 12.27 | . . . . . . . . . . . . . . . . . . . . . . . . | 34.73 |
| 12.29 | . . . . . . . . . . . . . . . . . . . . . . . . | 34.73 |
| 12.53 | . . . . . . . . . . . . . . . . . . . . . . . . | { 34.26 |
| 12.56 | . . . . . . . . . . . . . . . . . . . . . . . . | 34.26 |
| 2.15 | Absolutely no laking[2] . . . . . . . . . . . . . . . | { 34.92 |
| | | 34.77 |
| | | 34.77 |
| 2.45 | . . . . . . . . . . . . . . . . . . . . . . . . | { 34.73 |
| | | 34.73 |
| 4.48 | Absolutely no laking.[2] (Sediment 3) . . . . . . . . . . | { 35.36 |
| 4.50 | . . . . . . . . . . . . . . . . . . . . . . . . | 35.26 |
| Feb. 9 | | |
| A. M. | | |
| 9.38½ | . . . . . . . . . . . . . . . . . . . . . . . . | { 37.06 |
| 9.41 | . . . . . . . . . . . . . . . . . . . . . . . . | 36.90 |
| 9.43 | Very little laking. Not more than 1.8 per cent of the hæmoglobin in solution.[2] (Sediment 4) . . . . . . . . . | 36.95 |
| 10.01 | Control . . . . . . . . . . . . . . . . . . . . . | { 34.44 |
| 10.05 | No laking whatever.[2] (Sediment 5) . . . . . . . . . . | 34.49 |
| P. M. | | |
| 3.17 | Serum from A (after centrifugalizing for three hours contains some hæmoglobin) . . . . . . . . . . . . . . . . | 83.66 |
| | Serum from control (after centrifugalizing for three hours contains a little hæmoglobin, but less than in serum from A) | 82.57 |
| 3.46 | Sediment from A . . . . . . . . . . . . . . . . . . | { 6.98 |
| 3.47½ | . . . . . . . . . . . . . . . . . . . . . . . . | 6.94 |
| | | 6.91 |
| 4.07 | Sediment from control . . . . . . . . . . . . . . . | { 6.81 |
| 4.09 | . . . . . . . . . . . . . . . . . . . . . . . . | 6.69 |
| 4.10½ | . . . . . . . . . . . . . . . . . . . . . . . . | 6.55 |

[1] As in all the tables, λ is an abbreviation for $\lambda\ (0^\circ) \times 10^8$, the conductivity (at 0° C.) being expressed in reciprocal ohms.

[2] By centrifugalization test, 1 c.c. of the blood being mixed with 14 c.c. of ice-cold NaCl solution, and centrifugalized. "Sediment 3," etc., means that the sediment in the centrifuge tube was so numbered and set aside.

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MENT I.

mixture "control." At 3.12 P.M. added to 25 c.c. of defibrinated blood 1.5 c.c. of 2 per cent sapotoxin solution, both ice-cold. Call the mixture A′. Kept at room temperature. For the sapotoxin solution $\lambda = 90.22$; for the NaCl solution $\lambda = 88.95$; for the defibrinated blood $\lambda = 32.09$; for the serum from the clot, $\lambda = 73.05$ (Serum contains much fat).

| P. M. | | $\lambda$ |
|---|---|---|
| 3.20 | A′ . . . . . . . . . . . . . . . . . . . . . . . . | { 34.96 |
| 3.34 | To eye no laking whatever . . . . . . . . . . . . . . | } 34.77 |
| 3.59 | . . . . . . . . . . . . . . . . . . . . . . . . . . | { 36.74 |
| 4.02 | . . . . . . . . . . . . . . . . . . . . . . . . . . | } 36.74 |
| 4.03 | . . . . . . . . . . . . . . . . . . . . . . . . . . | ( 36.63 |
| 4.06 | Practically no hæmoglobin in solution, certainly less than 1 per cent of the whole.[1] (Sediment 1) . . . . . . | 36.63 |
| 4.30 | . . . . . . . . . . . . . . . . . . . . . . . . . . | { 36.79 |
| 4.31 | 3.5 per cent of hæmoglobin is now in solution.[1] (Sediment 2) | } 36.79 |
| Feb. 9 | | |
| A. M. | | |
| 10 30½ | . . . . . . . . . . . . . . . . . . . . . . . . . . | { 33.89 |
| 10.32 | . . . . . . . . . . . . . . . . . . . . . . . . . . | } 33.80 |
| 10.34½ | 54 per cent of the hæmoglobin in solution.[1] (Sediment 6) . | ( 33.71 |
| Feb. 10 | | |
| P. M. | | |
| 4.53 | . . . . . . . . . . . . . . . . . . . . . . . . . . | { 34.54 |
| 4.55 | . . . . . . . . . . . . . . . . . . . . . . . . . . | } 34.35 |
| | Even after twenty-four hours longer A′ is not completely laked.[1] | |

February 10.

2 P. M. Sediments 1 and 2 shaken up in NaCl solution and centrifugalized. Considerable laking. In 1, 41 per cent of hæmoglobin has gone into solution since sediment first obtained, and in 2 about 50 per cent. Some laking in 3, though not so much as in 2 (23 per cent of the hæmoglobin is in solution). In 4 practically no laking. (All kept at air temperature since first sedimentation.)

February 11.

2 P. M. In 1 and 2 laking has made much further progress, and only a small residue of unlaked corpuscles is now left, about the same in 1 as in 2. The same is true of 3, in which, however, the residue of unlaked corpuscles is a little greater than in 1 and 2.

The residue in 3 is not quite 0.1 c.c. In 4 laking has also advanced markedly, and residue is little greater than in 3. In 5 laking has advanced very little, and the residue of unlaked corpuscles amounts to 0.5 c.c.

In 6 laking has hardly advanced any further than yesterday. The amount of sediment in 6 is fully as great as in 4, being a little over 0.1 c.c. All these were kept at air temperature (19°).

A′ is not yet completely laked, as shown by centrifugalizing.

Control kept at air temperature is red, and shows no laking to eye.

[1] By centrifugalization test, 1 c.c. of the blood being mixed with 14 c.c. of ice-cold NaCl solution, and centrifugalized.

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## EXPERIMENT II.

October 21, 1902. — At 4.12 P.M. added to 50 c.c. dog's defibrinated blood drawn twenty-five hours ago and kept on ice, 3 c.c. of 2 per cent sapotoxin solution (in NaCl solution), the blood and solution having been previously cooled to 1.5° C. Call the mixture A. Kept in ice.

| P. M. | | λ |
|---|---|---|
| 4.18 | A | 25.63 |
| 4.20 | | 25.45 |
| 4.22½ | | 25.40 |
| 4.25 | | 25.40 |
| 5.12 | Another sample of A | 29.68 |
| 5.16 | | 29.68 |
| 5.25 | (A specimen of A centrifugalized after the addition of $FeCl_3$ [1] showed no laking) | 29.68 |
| 5.33 | | 29.51 |
| 5.39 | | 29.44 |
| 5.54 | Another sample of A | 29.27 |
| 6.00 | | 29.41 |
| 6.05 | | 29.41 |
| 6.10 | | 29.58 |
| 5.56 | A specimen of A centrifugalized after the addition of $FeCl_3$ showed distinct laking, about 40 per cent of the hæmoglobin having been liberated from the corpuscles, as shown colorimetrically. Twenty-two hours later A, which was kept in ice all through, was seen to be completely laked. | |
| 4.45 | Added to 5 c.c. of blood 0.3 c.c. of 0.9 per cent NaCl. | |
| 4.55 | This mixture | 26.08 |
| 4.58 | | 26.08 |
| 4.45 | Added to 5 c.c. of blood 0.3 c.c. of sapotoxin solution and left at room temperature. | |
| 5.05 | This mixture is much laked, though not completely. | |
| 6.27 | | 32.92 |
| 6.31 | | 32.89 |

[1] $FeCl_3$ was added in small amount to a measured quantity of the blood, according to the method of precipitating red corpuscles described by Dr. S. PESKIND. Then the mixture was rapidly shaken up with excess of ice-cold 0.9 per cent salt solution and centrifugalized.

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### EXPERIMENT III (1).

November 22, 1902.—At 4 P. M. added to 25 c.c. of dog's defibrinated blood drawn 3½ hours ago, and kept in ice, 1.5 c.c. of 2 per cent sapotoxin solution in 0.9 per cent NaCl solution, also ice-cold. Call mixture A. At 3.55 P. M. added to 10 c.c. of blood 0.6 c.c. of the sapotoxin solution. Call mixture A′. A was kept in ice, A′ at air temperature. At 4 P. M added to 10 c.c. of blood 0.6 c c. of 0.9 per cent NaCl solution. Call the mixture "control." Kept in ice.

For defibrinated blood . . . . . . . . $\lambda = 28.40$.
For sapotoxin solution . . . . . . . . $\lambda = 85.93$.
For the sodium chloride solution . . . . $\lambda = 87.41$.

| P. M. | | $\lambda$ |
|---|---|---|
| 4.20 | A . . . . . . . . . | 30.32 |
| 4.23 | . . . . . . . . . . | 30.18 |
| 4.27 | . . . . . . . . . . | 30.18 |
| 4.42 | . . . . . . . . . . | 30.18 |
| 5.05 | . . . . . . . . . . | 30.25 |
| 6.06 | . . . . . . . . . . | 31.89 |
| 6.10 | . . . . . . . . . . | 31.89 |
| 6.13 | Absolutely no laking[1] | 31.85 |
| 5.24 | Control . . . . . | 29.79 |
| 5.28 | . . . . . . . . . . | 29.72 |
| Nov. 23 | | |
| A. M. | | |
| 10.34 | A . . . . . . . . | 33.31 |
| 10.38 | No laking[1] . . . . | 33.31 |
| P. M. | | |
| 1.44 | . . . . . . . . . . | 33.09 |
| 1.48 | No laking[1] . . . . | 33.09 |
| Nov. 24 | | |
| P. M. | | |
| 4.49 | About 15 per cent of the hæmoglobin is now in solution.[1] | 31.41 |
| 4.51 | . . . . . . . . . . | 31.37 |
| | A was now placed at room temperature for twenty-four hrs., at end of which time $\lambda = 29.61$, the greater part of the hæmoglobin being now in solution. | |

| P. M. | | $\lambda$ |
|---|---|---|
| 6.30 | A′ (30 per cent laked)[1] | 36.21 |
| 6.35 | . . . . . . . . . . | 35.16 |
| 6.38 | . . . . . . . . . . | 35.01 |
| Nov. 23 | | |
| A. M. | | |
| 11.02 | . . . . . . . . . . | 33.09 |
| 11.17 | 50 per cent laked[1] . . | 32.96 |
| Nov. 24 | | |
| P. M. | | |
| 4.50 | Is not yet completely laked. Apparently about the same amount of laking as yesterday. | |
| | Twenty-four hrs. later (greater part of hæmoglobin is now in solution) . . . . | 29.79 |

[1] Determined by addition of $FeCl_3$ and cold NaCl solution and centrifugalizing.

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### EXPERIMENT III (2).

November 23.—At 11.35 A. M. added to 25 c.c. of same defibrinated blood 2.5 c.c. of sapotoxin solution, both ice-cold. Call mixture A. At 12.25 added to 10 c.c. of blood 1 c.c. of sapotoxin solution. Call mixture A′. Kept A in ice, A′ at air temperature. At 12.30 added to 10 c.c. of blood 1 c.c. of 0.9 per cent NaCl solution. Call the mixture "control." Kept in ice.

| A. M. | | λ | P. M. | | λ |
|---|---|---|---|---|---|
| 11.48 | A . . . . . . . . . | 34.30 | 1.00 | A′ completely laked. | |
| 11.51 | . . . . . . . . . . | 34.54 | 1.26 | . . . . . . . . . . | 46.34 |
| 11.55 | . . . . . . . . . . | 34.82 | 1.29 | . . . . . . . . . . | 46.34 |
| 11.57½ | . . . . . . . . . . | 34.87 | | | |
| P. M. | | | | | |
| 12.05 | . . . . . . . . . . | 35.21 | | | |
| 12.08 | No laking[1] . . . . | 35.16 | | | |
| 1.05 | Now there is obvious laking. | | | | |
| 1.08 | . . . . . . . . . . | 38.58 | | | |
| 1.10 | Most of the corpuscles are laked[1] . . . . | 38.53 | | | |
| 12.50 | "Control" . . . . | 33.22 | | | |

[1] Determined by addition of $FeCl_3$ and cold NaCl solution and centrifugalizing.

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## EXPERIMENT IV.

October 16, 1902. — At 4.25 P. M. added to 5 c.c. of dog's defibrinated blood drawn an hour ago 0.4 c.c. of a 2 per cent sapotoxin solution in 0.9 per cent NaCl solution, both cooled to 0° C. Kept the mixture in ice and made conductivity measurements at 0°.

For the sapotoxin solution . . . . $\lambda = 88.95$.
For the 0.9 per cent NaCl solution . $\lambda = 88.64$.

| P. M. | | $\lambda$ |
|---|---|---|
| 4.35 | Mixture of blood and sapotoxin . . . . . . . . . . . . | 28.81 |
| 4.41 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 29.82 |
| 4.46 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 29.96 |
| 4.51 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 29.68 |
| 4.57 | Shook up U tube . . . . . . . . . . . . . . . . . . . | 29.58 |
| 5.03 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 33.35 |
| 5.07 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 33.66 |
| Oct. 17 | | |
| 3.35 | 5 c.c. blood + 0.4 c.c. of 0.9 per cent NaCl (mixed twenty-four hours ago and kept on ice) . . . . . . . . . . . | 29.34<br>29.27 |
| 3.08 | The original defibrinated blood, kept in ice . . . . . . . . | 24.61 |
| 3.11 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 24.52 |
| 3.13 | . . . . . . . . . . . . . . . . . . . . . . . . . . . | 24.37 |

5 c.c. defib. blood + 0.4 c.c. sapotoxin, kept at 17° C., laked completely in 5 min.
5 c.c. defib. blood + 0.3 c.c. sapotoxin, kept at 17° C., laked in 20 min.
5 c.c. defib. blood + 0.2 c.c. sapotoxin, kept at 17° C., laked in 2½ hrs.
5 c.c. defib. blood + 0.3 c.c. sapotoxin, at 0° C., almost completely laked in 21 hrs.
5 c.c. defib. blood + 0.2 c.c. sapotoxin, at 0° C., only slightly laked in 21 hrs.

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### EXPERIMENT V.

October 18, 1902. — At 3.33 P. M. added to 15 c.c. dog's defibrinated blood (obtained forty-eight hours ago and kept in ice-chest) 0.9 c.c. sapotoxin. Kept mixture in ice. Call mixture A. For the sapotoxin solution $\lambda = 88.95$; for the 0.9 per cent NaCl $\lambda = 88.64$.

| P. M. | | $\lambda$ |
|---|---|---|
| 3.38 | A . . . . . . . . . . . . . . . . . . . . . . . . . | 25.12 |
| 3.41 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 25.40 |
| 3.45 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 26.00 |
| 3.50 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 26.97 |
| 3.55 | Not laked . . . . . . . . . . . . . . . . . . . . . . | 27.95 |
| | Now took out U tube and left in air ten minutes, then put back in ice. | |
| 4.09 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 29.82 |
| 4.13 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 30.25 |
| 4.17 | The blood is now pretty well laked . . . . . . . . . . . | 30.58 |
| 4.39 | Refilled U tube from stock of A kept in ice all the time since mixture . . . . . . . . . . . . . . . . . . . . | 31.03 |
| 4.41 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 31.10 |
| 4.45 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 31.22 |
| 4.48 | Not laked . . . . . . . . . . . . . . . . . . . . . . | 31.30 |
| | Took out U tube from ice, heated in hand, shook up and put back in ice. Blood is now somewhat darker, though not by any means completely laked. | |
| 5.02 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 31.07 |
| 5.05 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 31.93 |
| 5.11 | Now fairly well laked . . . . . . . . . . . . . . . . . | 32.92 |
| 3.45 | Added to 15 c.c. defibrinated blood 0.9 c.c. sapotoxin, and left at air temperature (18°). | |
| 4.10 | Completely laked. | |
| 5.20 | . . . . . . . . . . . . . . . . . . . . . . . . | 36.47 |
| 5.23 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 36.47 |
| 3.33 | Added to 15 c.c. defibrinated blood 0.9 c.c. of the 0.9 per cent NaCl solution. | |
| 5.33 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 24.66 |
| 5.35 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 24.61 |
| 5.50 | Refilled U tube from stock of A kept in ice (does not seem laked) . . . . . . . . . . . . . . . . . . . . | 31.10 |
| 5.54 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 31.22 |
| 5.56 | . . . . . . . . . . . . . . . . . . . . . . . . . . | 31.18 |

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## EXPERIMENT VI.

January 11. — To 1 c.c. of dog's corpuscles, washed thoroughly and suspended in 0.9 per cent NaCl, added 0.04 c.c. of 2 per cent sapotoxin solution in 0.9 per cent NaCl. Immediate laking. For the suspension, λ = 13.78; for the NaCl solution, λ = 89.26; percentage of corpuscles in suspension, calculated by the electrical method, 75 per cent. At 11.13 A. M. added to 10 c.c. of the suspension 0.2 c.c. of the sapotoxin solution, both ice-cold, and kept the mixture in ice. It begins to lake almost immediately.

| | | λ | |
|---|---|---|---|
| 11.21 | Mixture . . . . . . . | 16.35 | |
| 11.24 | . . . . . . . . . . . . | 16.68 | |
| | After 20 hrs. at 8–10° . . . | 31.89 | Microscopically: No intact corpuscles, but numerous swollen leucocytes and hæmoglobin crystals. |
| | At 11.45 added to 5 c.c. of the suspension 0.07 c.c. of the sapotoxin solution, both ice-cold, and kept the mixture in ice. | | |
| 11.52 | The mixture . . . . . . | 9.90 | |
| 12.12 | 58 per cent of the hæmoglobin is in solution[1] . . . | 10.31 | Microscopically examined at once; many hæmoglobin-containing corpuscles, mostly round, but some crenated. Some ghosts. On standing until next morning, few or no unaltered corpuscles. |
| | At 12.40 added to 5 c.c. of the suspension 0.3 c.c. of a 0.2 per cent sapotoxin solution in 0.9 per cent NaCl solution, both ice-cold, and kept the mixture in ice. | | |
| 12.43 | Mixture . . . . . . . | 15.65 | |
| 12.45 | . . . . . . . . . . . . | 14.40 | |
| 12.50 | . . . . . . . . . . . . | 12.67 | |
| 12.56 | 24 per cent of hæmoglobin is in solution[1] . . . . | 11.63 | Microscopically: Numerous corpuscles containing hæmoglobin, mostly round, but some crenated, and some ghosts. On standing until next morning, few or no unaltered corpuscles. |

[1] Sediment was repeatedly washed in cold NaCl solution to remove all sapotoxin in the solution. Evidently the corpuscles had fixed some sapotoxin, since they laked partially on standing till next morning at air temperature, while a control specimen of blood containing no sapotoxin did not lake. The washed sapotoxin corpuscles did not lake so completely as corpuscles from the same mixture left still in contact with the salt solution containing sapotoxin.

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EXPERIMENT VII.

January 20. — Dog's defibrinated blood obtained January 18 at noon. Washed twice in 0.9 per cent NaCl solution in the cold.

1 c.c. of the suspension plus 0.1 c.c. of 0.2 per cent sapotoxin solution in 0.9 per cent NaCl solution lakes at once at air temperature.

1 c.c. of the suspension plus 0.05 c.c. of 0.2 per cent sapotoxin solution lakes partially in one minute at air temperature. In one hour not completely laked. Completely laked next morning at 10 A. M.

1 c.c. of the suspension plus 0.03 c.c. of 0.2 per cent sapotoxin solution. No laking in five minutes. In twelve minutes is a little darkened. In one hour distinctly darkened, but not by any means completely laked. Completely laked next morning at 10 A. M.

For the suspension $\lambda = 46.86$ on January 20 and 46.43 on January 21.

At 3.03 P. M. added to 20 c.c. of the suspension 0.8 c.c. of 0.2 per cent sapotoxin. Kept at air temperature (18° to 20° C.). Call the mixture A′. At 3.16 added to 20 c.c. of the suspension 0.8 c.c. of 0.2 per cent sapotoxin, both ice-cold, and kept in ice. Call the mixture A. At 4.10 added to 20 c.c. of the suspension 0.8 c.c. of 0.9 per cent NaCl, and kept in ice. Call the mixture "control."

For the 0.9 per cent NaCl solution $\lambda = 89.26$
For the 0.2 per cent sapotoxin . . $\lambda = 89.26$
For the defibrinated blood $\lambda = 29.41$
Serum from clot . . . . $\lambda = 71.01$

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EXPERIMENT VII (*continued*).

| P. M. | | λ | P. M. | | λ |
|---|---|---|---|---|---|
| 3.22 | A . . . . . . . . . | 48.94 | 3.09 | A′ is somewnat darkened. | |
| 3.25 | . . . . . . . . . . | 48.29 | 3.30 | Very distinctly darkened. | |
| 3.30 | . . . . . . . . . . | 47.47 | | Eighteen and one-half hrs. later completely laked. | |
| 3.33 | Slight laking, 8 per cent of the hæmoglobin in solution.[1] (Sed. 1) . | 47.21 | 5.24 | . . . . . . . . . . | 40.56 |
| 3.52 | . . . . . . . . . . | 45.60 | 5.26 | . . . . . . . . . . | 40.50 |
| 3.56 | . . . . . . . . . . | 45.51 | 5.27 | 57 per cent of hæmoglobin in sol.[1] (Sed. 5) | 40.50 |
| 3.58 | 16 per cent of the hæmoglobin in solution.[1] (Sediment 2) | 45.51 | Jan. 21 | | |
| 5.02 | . . . . . . . . . . | 45.27 | 3.20 | 64 per cent of hæmoglobin in sol.[1] (Sed. 8) | 40.50 |
| 5.05 | . . . . . . . . . . | 45.19 | | 24 hours later . . . | 45.19 |
| 5.08 | 20 per cent of the hæmoglobin in solution.[1] (Sediment 4) | 45.03 | Jan. 22 | | |
| Jan. 21 | | | 3.15 | To 5 c.c. of A′ added 0.2 c.c. of 2 per cent sapotoxin. Lakes completely. In sediment many leucocytes and granules, but no ghosts. | |
| 2.30 | . . . . . . . . . . | 45.51 | | | |
| 2.35 | 43 per cent of the hæmoglobin in solution.[1] (Sediment 6) | 45.43 | | | |
| Jan. 20 | | | | | |
| 4.40 | Control . . . . . | 48.01 | | | |
| 4.42 | . . . . . . . . . . | 47.92 | 3.33 | This mixture . . . . | 66.01 |
| 4.46 | No laking.[1] (Sed. 3) | 47.83 | 3.53 | A′ plus as much of 0.9 per cent NaCl as was added of the 2 per cent sapotoxin sol. . | 47.83 |
| Jan. 21 | | | | | |
| 2.59 | Slight laking.[1] (Sed. 7) | 46.43 | | | |

Microscopically, A′; numerous ghosts perfectly round. Some corpuscles still contain some hæmoglobin, although all are altered and swollen. None are crenated.

[1] Tested by centrifugalization method. "Sediment 1," etc., means that the sediment in the centrifuge tube was so numbered and set aside. At 5.30 P. M., on January 20, sediments 1, 2, 3, 4, and 5 were washed twice in ice-cold salt solution (each time in 16 c.c.) and then left at room temperature. Considerable laking took place in all except 3.

January 21 at 4.20 P. M. heated sediments 1, 2, 3, 4, 5, 6, 7, and 8 to 45° C. (after washing twice in NaCl solution), then made up to 15 c.c. with NaCl solution and centrifugalized.

In 3 and 7 some laking has taken place, but much less than in the others. In 1, sediment is somewhat greater than in 2; in 2, two or three times as great as in 4; in 4 and 5, about the same, the supernatant liquid in 5 being less deeply tinged than in 4; in 6, sediment is not half as great as in 5, and about equal to that in 8, the supernatant liquid in 8 being less deeply tinted than in 6.

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EXPERIMENT VIII.

October 27, 1902. — Made a suspension of washed formaldehyde-fixed dog's corpuscles in 0.9 per cent NaCl. Call it A.

For the 0.9 per cent NaCl solution . . . $\lambda = 87.11$.
For the sapotoxin solution . . . . . . . $\lambda = 85.64$.

| P. M. | | $\lambda$ |
|---|---|---|
| 4.52 | A . . . . . . . . . . . . . . . . . . . . . . . . | 53.37 |
| 4.56 | . . . . . . . . . . . . . . . . . . . . . . . . . | 52.81 |
| 4.55 | Added to 10 c.c. of A 0.6 c.c. of sapotoxin solution. Kept at air temperature. | |
| 5.03 | This mixture . . . . . . . . . . . . . . . . . . . . . | 71.61 |
| 5.10 | . . . . . . . . . . . . . . . . . . . . . . . . . | 71.21 |
| 5.13 | . . . . . . . . . . . . . . . . . . . . . . . . . | 70.03 |
| 6.15 | Refilled U tube . . . . . . . . . . . . . . . . . . . . | 71.01<br>71.41 |
| 5.07 | Added to 10 c.c. of A cooled to 0° C., 0.6 c.c. of ice-cold sapotoxin solution. Kept the mixture in ice. | |
| 5.24 | This mixture . . . . . . . . . . . . . . . . . . . . . | 69.45 |
| 5.30 | . . . . . . . . . . . . . . . . . . . . . . . . . | 69.45 |
| 5.57 | Refilled U tube . . . . . . . . . . . . . . . . . . . . | 69.83<br>70.03 |
| 5.12 | Added to 5 c.c. of A 0.3 c.c. of 0 9 per cent NaCl. Kept mixture at air temperature. | |
| 5.42 | . . . . . . . . . . . . . . . . . . . . . . . . . | 54.05 |

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## EXPERIMENT IX.

December 16, 1902. At 2.52 P. M. added to 10 c.c. of dog's defibrinated blood obtained 24 hours ago and kept in ice, 2 c.c. of 2 per cent Na taurocholate in 0.9 per cent NaCl, both ice-cold. Call the mixture A. A is kept in ice. At 3.40 added to 10 c.c. of the defibrinated blood, 2 c.c. of the taurocholate solution and kept at room temperature. Call the mixture A′. At 2.58 added to 10 c.c. of the defibrinated blood, 2 c.c. of 0.9 per cent NaCl. Call the mixture "control." Kept in ice. To 10 c.c. of the blood added 2 c.c. of a NaCl solution whose $\lambda$ was 97.12. Call the mixture "second control."

For defibrinated blood . . . . . . . $\lambda = 29.51$.
For the serum from the clot . . . . . $\lambda = 71.61$.
For the taurocholate solution . . . . . $\lambda = 96.75$.
For the 0.9 per cent NaCl solution . . . $\lambda = 91.20$.

| P. M. | | λ | P. M. | | λ |
|---|---|---|---|---|---|
| 3.08 | A . . . . . . . . | 40.56 | 4.42 | A′ (partially laked). 28 per cent of hæmoglobin being in solution.[1] | 32.21 |
| 3.16 | . . . . . . . . . | 40.43 | | | |
| 4.55 | . . . . . . . . . | 39.55 | | | |
| 5.00 | A is now slightly laked, 7.7 per cent of the hæmoglobin being in solution.[1] | | Dec. 17 A. M. 10.00 | A′ is now completely laked. | |
| Dec. 17 | | | P. M. 4.22 | A′ (shows no sediment) . . . . . | 33.22 |
| 3.30 | Serum of A (separated by centrifug. and containing more hæmoglobin than last night) | 47.65 | 4.24 | . . . . . . . . | 33.27 |
| 3.44 | Sediment of A . . | 20.61 | | | |
| 4.00 | A . . . . . . . . | 36.21 | | | |
| Dec. 16 | | | | | |
| 3.48 | Control . . . . . | 38.06 | | | |
| | Serum from control (contains trace of hæmoglobin in solution) . . . . | 79.47 | | | |
| | Second control . . | 39.42 | | | |

[1] Estimated by diluting with ice-cold NaCl solution and centrifugalizing.

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## EXPERIMENT X.

December 20. 9.35 A.M. To 1 c.c. of a sediment of dog's corpuscles washed free from serum by NaCl solution, added 0.1 c.c. of 2 per cent Na taurocholate solution in 0.9 per cent NaCl, and kept at air temperature. In one hour partial laking has occurred. At 2 P.M. it is not yet completely laked. To 1 c.c. of the sediment added 0.2 c.c. of taurocholate solution. It begins to darken in a few minutes. In an hour it is much darkened. At 2 P.M. well laked. At 10.40 A.M. added to 10 c.c. of the sediment 1 c.c. of the taurocholate solution, both ice-cold, and kept in ice. Call the mixture A. At 11 A.M. added to 4 c.c. of the sediment 0.4 c.c. of a NaCl solution, whose $\lambda = 96.39$. Kept in ice. Call this mixture "control."

For the sediment . . . . . . . $\lambda = 24.10$
For the taurocholate solution . . $\lambda = 97.12$

| A.M. | | λ | A.M. | | λ |
|---|---|---|---|---|---|
| 10.54 | A . . . . . . . . | 26.41 | 11.23 | Control . . . . | 29.14 |
| 10.57 | . . . . . . . . . | 26.41 | 11.25 | . . . . . . . . . | 29.14 |
| 11.45 | . . . . . . . . . | 26.97 | 12.20 | . . . . . . . . . | 28.58 |
| 11.47½ | Partially laked; 40% of the hæmoglobin is in solution | 26.97 | 12.21 | . . . . . . . . . | 28.51 |
| 12.00 | Took A out of ice and left at room temperature. | | | | |
| P.M. 2.00 | Much darkened, though not completely laked. | | | | |
| 2.32 | . . . . . . . . . | 20.95 | | | |
| 2.35 | 70% of the hæmoglobin is now in solution . . . . | 20.95 | | | |

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## EXPERIMENT XI.

November 24. — Added to rabbit's blood, obtained twenty-four hours previously and kept in ice, an equal volume of dog's serum obtained from clot drawn sixty-nine hours before, and kept in ice. Complete laking at room temperature in three and one-half hours. (No doubt it was laked before this.) A control with ten parts of dog's serum (heated to 56° C. for forty-five minutes, and to 60° C. for five minutes) added to 1 part of rabbit's blood, showed no laking in three and one-half hours.

For rabbit's blood . . . . . . . . . . $\lambda = 44.05$
For unheated dog's serum . . . . . . $\lambda = 76.59$

At 3.50 P. M. added to 5 c.c. rabbit's blood 5 c.c. of heated dog's serum. Call mixture A′. Kept in ice. At 3.55 added to 15 c.c. rabbit's blood 15 c.c. of unheated dog's serum (both previously cooled to 0°). Call mixture A.

| P. M. | | λ | P. M. | | λ |
|---|---|---|---|---|---|
| 3.55 | A . . . . . . . | 58.54 | 4.33 | A′. . . . . . . . | 58.54 |
| 4.15 | . . . . . . . . . | 58.41 | 4.35 | . . . . . . . . . | 58.54 |
| 4.17 | . . . . . . . . . | 58.01 | | | |
| 5.19 | . . . . . . . . . | { 58.28 | | | |
| 5.20 | . . . . . . . . . | } 58.14 | | | |
| Nov. 25 | | | Nov. 25 | | |
| 3.31 | Some laking . . . | { 58.54 | 4.12 | No laking. | |
| 3.34 | . . . . . . . . . | } 57.61 | 4.15 | . . . . . . . . . | 58.68 |
| 3.40 | . . . . . . . . . | ( 58.14 | 4.17 | . . . . . . . . . | 58.28 |

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## SUMMARY.

1. At 0° C the laking action of sapotoxin is much retarded, and it can be shown that before any hæmoglobin has been liberated the conductivity of the blood is increased, presumably owing to an increase in the permeability of the envelopes of the corpuscles to electrolytes. At this stage sapotoxin has been fixed by the corpuscles. Bile salts produce the phenomenon less distinctly, foreign serum not at all.

2. Both at 0° and at ordinary temperature a dose of sapotoxin just sufficient to cause liberation of the hæmoglobin causes the discharge of only a small proportion of the electrolytes of the corpuscles. When the dose is increased the electrolytes are discharged.

3. It seems permissible to divide the action of sapotoxin on the corpuscles into three stages: (1) an action on the envelope, which does not necessarily nor immediately lead to the liberation of the hæmoglobin; (2) an action on the hæmoglobin or the stroma which causes the discharge of the pigment; (3) an action on the stroma leading to the setting free of electrolytes.

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# QUICK METHODS FOR CRYSTALLIZING OXYHÆMOGLOBIN: INHIBITORY AND ACCELERATOR PHENOMENA, ETC.: CHANGES IN THE FORM OF CRYSTALLIZATION.

By EDWARD T. REICHERT.

## I. Quick Methods for Crystallizing Oxyhæmoglobin.

THE most expeditious known method for preparing crystals of the oxyhæmoglobin of the blood of the dog is to lake the blood with ether, and subject it to cold. Crystals appear within a variable period, ranging from a few minutes to many hours. I have found that if to the blood there be added, before or after laking, from 1 to 5 per cent of ammonium oxalate, crystallization invariably begins immediately, and that any quantity of crystals can be obtained within a few hours at ordinary room temperature. If a drop of this blood be placed under the microscope, crystals will be seen to form at once near the margin of the drop, and to be deposited so rapidly that a solid mass is formed in a few minutes.

The blood of the horse does not yield so readily to this treatment. If a drop of blood so prepared be examined under the microscope, it will be found that crystallization will not begin until after from fifteen to forty minutes or more, and that it will proceed slowly. Better results can be obtained if the blood be oxalated and set aside for the corpuscles to subside. The supernatant liquid is then poured off, and the remainder laked with ether.

Defibrinated blood of the rat, laked with water on a slide, and covered with a cover-glass after the margin of the drop has become dried, usually crystallizes very readily, as is well-known. Better results can be obtained if the blood be oxalated before or after laking; and even more rapid crystallization occurs if the blood be laked with ether instead of water. Crystals form so rapidly in the oxalate-ether blood that a magma is formed in the test-tube within a few minutes.

The oxalate-ether process applied to the blood of the guinea-pig gives most satisfactory results. Crystallization does not proceed



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quite so rapidly as in rat's blood, yet within a minute or two innumerable tetrahedra appear, and any quantity can be obtained within a couple of hours.

The blood of the necturus crystallizes readily when so treated, which is a matter of especial interest because of the difficulty heretofore experienced in obtaining crystals of oxyhæmoglobin from the blood of cold-blooded animals. The crystals resemble those of the triple phosphates.

The rapidity with which crystallization begins and proceeds is influenced decidedly both by the method of laking and the percentage of oxalate. Ethyl ether is a much better laking agent than water, and acetic ether is stronger than ethyl ether. The presence of any quantity of oxalate up to saturation increases crystallizability, but I have thus far found from 1 to 5 per cent to be the best; the larger the quantity the more is crystallization hastened. When more than 5 per cent is used, the oxalate also tends to crystallize upon the slide. If the blood be prevented from drying, as in the test-tube, the oxalate remains in solution.

Asphyxial blood yields crystals more readily than normal blood.

## II. Inhibitory and Accelerator Phenomena, etc.

If to the blood of one species, the blood, plasma, or serum of another species be added, the laking of the blood may be retarded, accelerated, or unaffected, according to the character of the mixture. The period required for laking may be prolonged for five minutes or more.

The crystallization of the oxyhæmoglobin may be hindered or prevented in such mixtures. Thus, by varying the proportions of a mixture of the bloods of the dog and guinea-pig, crystals from one or both may appear, but the process is invariably retarded, and sometimes to a marked degree. If crystals of both kinds of oxyhæmoglobin are deposited, those of one usually begin forming some time before those of the other, and the crystallization of one may be complete before crystals of the other are seen.

## III. Changes in the Form of Crystallization.

The typical forms of the crystals of certain kinds of oxyhæmoglobin may be modified or completely changed when the bloods of two species are mixed. Thus, if to the blood of the rat there be added a definite percentage of the blood of the guinea-pig, crystals of the rat's

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oxyhæmoglobin may appear in unaltered form, but most, if not all, of those from the guinea-pig's blood will be changed; in fact, if any perfect tetrahedra are found, they will have been formed at the very end of the crystallization. If the proportions of the mixture be properly modified, not a single crystal of what can be identified as rat's oxyhæmoglobin will appear, and all of the crystals will be modified tetrahedra and *spindles*, and transitional forms between these. The spindles resemble Charcot's crystals in form but not in color; they vary in size, some of them being very large, and some may have small spindles attached to them; and they can be obtained having sharp angles, if crystallization has not been too rapid.

This complete change in the form of the crystals of oxyhæmoglobin, when the bloods of two species are mixed, and the spindle-shaped form of the crystals, are, I believe, unique facts in the crystallography of this most important substance.

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# ARTIFICIAL PARTHENOGENESIS IN NEREIS.

By MARTIN H. FISCHER.

[*From the Physiological Laboratory of the University of California.*]

LAST year Dr. Loeb and I[1] published a note in this journal in which we stated that it is possible to cause the unfertilized eggs of Nereis limbata to develop into swimming larvæ by keeping them for some time in sea-water, the concentration of which has been raised by the addition of potassium chloride, and then returning them to ordinary sea-water. We expressed the opinion that we were probably dealing with osmotic effects, and not with the specific effects of K-ions, since we obtained swimming larvæ only from those mixtures of sea-water and potassium chloride in which the osmotic pressure had been considerably raised. We based our conclusions upon a series of experiments on the eggs of a single female, but did not publish them, as we were not able to obtain any more mature females upon which to corroborate our findings. This year we were more fortunate, however, and succeeded both in repeating our experiments of last year, and of obtaining further proof of the correctness of our conclusions.

All the precautions necessary to prevent the infection of the eggs with sperm, which have been so often described by Loeb,[2] were followed in these experiments. Each female Nereis, as soon as caught, was kept in a separate dish of sea-water sterilized by heating to 80°C. After swimming about in this for some time, the worms were washed in several changes of sea-water, then in fresh water, and finally were opened in sterile sea-water with sterilized instruments.

I shall give first of all a detailed description of the series of experiments which formed the subject of our first communication.

[1] FISCHER, M. H.: This journal, 1902, vii, p. 313; LOEB, FISCHER, and NEILSON: Archiv für die gesammte Physiologie, 1901, lxxxvii, p. 594.

[2] LOEB, J.: This journal, 1901, iv, p. 424.



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*Experiment I, July 20, 1901.* — 12.30 A. M. The eggs of two females were gently shaken together and distributed into the following mixtures of potassium chloride and sterile sea-water: —

1. 95 c.c. sea-water + 5 c.c. KCl $2\frac{1}{2}$ *m*.
2. $92\frac{1}{2}$ c.c. sea-water + $7\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m*.
3. 90 c.c. sea-water + 10 c.c. KCl $2\frac{1}{2}$ *m*.
4. $87\frac{1}{2}$ c.c. sea-water + $12\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m*.
5. 85 c.c. sea-water + 15 c.c. KCl $2\frac{1}{2}$ *m*.
6. $82\frac{1}{2}$ c.c. sea-water + $17\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m*.
7. 80 c.c. sea-water + 20 c.c. KCl $2\frac{1}{2}$ *m*.
8. $77\frac{1}{2}$ c.c. sea-water + $22\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m*.
9. 75 c.c. sea-water + 25 c.c. KCl $2\frac{1}{2}$ *m*.
10. $72\frac{1}{2}$ c.c. sea-water + $27\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m*.
11. 70 c.c. sea-water + 30 c.c. KCl $2\frac{1}{2}$ *m*.
12. Sterile sea-water (control).

The eggs were taken out of the solutions and transferred to sterile sea-water at intervals of thirty, forty-five, and sixty minutes. These will be designated as Lots I, II, and III.

The first evidences of segmentation were found in the eggs exposed for thirty minutes to the mixture of 80 c.c. sea-water + 20 c.c. KCl $2\frac{1}{2}$ *m*. One hour and twenty minutes after returning the eggs to sea-water several were found in the two-cell stage. Ten minutes later an egg was found in the four-cell stage. After two hours, with rare exceptions, all the dishes contained segmenting eggs. In some of the dishes the eggs did not pass through the two and four-celled stages, but broke up immediately into eight and twelve cells. Up to five hours after the beginning of the experiment, it was difficult to decide which salt solution would give the most favorable results. After ten and a half hours (10.55 A. M.), however, it was clearly noticeable that the optimum sea-water-salt solution mixture lay between $77\frac{1}{2}$ c.c. sea-water + $22\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m* and 85 c.c. sea-water + 15 c.c. KCl $2\frac{1}{2}$ *m*. All these solutions contained eggs in the thirty-two to sixty-four cell stages.

The first swimming larvæ were found in the afternoon, fourteen hours after the return of the eggs to sea-water, in Lot III of the mixture of $82\frac{1}{2}$ c.c. sea-water + $17\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m*. Lots II and I showed the same condition of affairs. In one field of the latter every egg was in the blastula stage, and many were beginning to swim.

At four o'clock, Lot I of the mixture of 80 c.c. sea-water + 20 c.c. KCl $2\frac{1}{2}$ *m* showed even better results than the foregoing, the dish teeming with swimming larvæ. In Lots II and III of the same mixture many less were found. Lots II and III of $77\frac{1}{2}$ c.c. sea-water + $22\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m* showed some swimming larvæ. The solutions containing a greater amount of KCl than this showed many segmented eggs but no swimming

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larvæ. In solutions less concentrated than 85 c.c. sea-water + 15 c.c. KCl 2½ *m*, up to this time, no swimming larvæ had developed. A few hours later occasional swimming larvæ were found in these solutions also.

At 1.00 A. M., twenty-four hours after the return of the eggs to sea-water, with an occasional exception, every egg from Lot I of the mixture of 80 c.c. sea-water + 20 c.c. KCl 2½ *m* was swimming. In Lot I of 82½ c.c. sea-water + 17½ c.c. KCl 2½ *m* many were swimming. In the rest of the dishes all were dead.

The control eggs left in the sterile sea-water contrasted sharply with those which had been in the sea-water-salt solution mixtures. Not a single egg segmented throughout the night. Eighteen hours after the beginning of the experiment a few were found in the two to four cell stage. Six hours later, when the other dishes were teeming with swimming larvæ, the control eggs were granular and surrounded by a granular débris. The dead eggs floated in the water, and were held together by a gelatinous material. Not a single swimming larva developed.

Many of the swimming larvæ lived through the next day. One was removed to fresh sea-water in a separate dish. This embryo lived fifty-seven hours.

*Experiment II, July 6, 1902.* — 11.05 P. M. It was necessary, first of all, to repeat the experiment of last year. I distributed the eggs of a freshly caught Nereis into the following solutions:

1. 25 c.c. KCl 2½ *m* + 75 c.c. sea-water.
2. 22½ c.c. KCl 2½ *m* + 77½ c.c. sea-water.
3. 20 c.c. KCl 2½ *m* + 80 c.c. sea-water.
4. 17½ c.c. KCl 2½ *m* + 82½ c.c. sea-water.
5. 15 c.c. KCl 2½ *m* + 85 c.c. sea-water.
6. 12½ c.c. KCl 2½ *m* + 87½ c.c. sea-water.
7. 10 c.c. KCl 2½ *m* + 90 c.c. sea-water.
8. 7½ c.c. KCl 2½ *m* + 92½ c.c. sea-water.
9. 5 c.c. KCl 2½ *m* + 95 c.c. sea-water.
10. 2½ c.c. KCl 2½ *m* + 97½ c.c. sea-water.
11. Sterile sea-water (control).

I removed one lot of eggs at 12.00 midnight, a second at 12.25 A. M. These will be designated as Lots I and II.

At 3.00 A. M. July 7, every egg in the control was intact. A few eggs were removed from Lot I of Solution 4 for microscopic study. These were somewhat shrunken and opaque, had lost their nuclei, and no longer showed the peripheral arrangement of the oil globules which is so characteristic of the unfertilized egg. At 4.30 A. M. I found the same condition of affairs in Lot II of Solution 5. The eggs were fissured and broken up into irregular masses (cells?). Only occasionally were these arranged regularly as in the normal cleavage of the fertilized egg.

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At 9.00 A. M. conditions were much the same, except that cleavage had gone further. At 10.30 I noticed that many of the lines of cleavage had become obscure or had disappeared entirely, so that the eggs were again spherical. The control eggs were still intact. At 11.30 A. M., however, a few were found in which the peripheral arrangement of the oil globules was lost. The eggs from Solutions 7 and 8 were nearly all intact. A few were somewhat shrunken, irregular in outline, and had lost the peripheral arrangement of their oil globules.

At 9.30 P. M. conditions were as follows: Not a single swimming larva was found in the control. Some of the eggs had undergone a granular degeneration and were going to pieces. A few had lost the peripheral arrangement of the oil globules; the remainder were intact, and looked as though they had just been removed from the ovaries. Both lots of Solutions 1 and 2 yielded no swimming larvæ. The eggs were irregular and contracted, with thick, clear membranes. Many were dying, and no traces of segmentation could be found in Lot II of Solution 1. Several swimming larvæ were obtained in both lots removed from Solution 3. The optimum salt-solution sea-water mixture was Solution 4. This yielded many swimming larvæ, as did also Solution 5. A few swimming larvæ were found in Lot II of Solutions 6 and 7. One swimming larva was found in Lot II of Solution 8, and one in Lot II of Solution 9. An occasional egg, segmented into two or four cells, was found in Solution 10.

At noon, July 9, 1902, larvæ were still swimming in the dishes containing the eggs from Solutions 3, 4, and 5. The control eggs still showed the peripheral arrangement of the oil globules, or else were broken up into a granular débris. In the other dishes, all were dead. I kept the control eggs for another day, but no swimming larvæ developed.

The experiment seemed to leave no doubt as to the correctness of the findings of last year. The unfertilized eggs of Nereis, if left undisturbed in sea-water, will not develop into swimming larvæ. After several hours, however, they may show the changes which precede cleavage, or may actually divide into two or four cells. If the eggs are, however, kept for an hour in a mixture of $17\frac{1}{2}$ c.c. KCl $2\frac{1}{2}$ *m* + $82\frac{1}{2}$ c.c. sea-water, and are then removed to ordinary sea-water, they will develop into swimming larvæ.

*Experiment III, July 8, 1902.* — 2.10 A.M. I wished once more to assure myself of the correctness of the results obtained in the last experiment; I wished also to discover whether the KCl acted only through its osmotic effects. The following solutions were therefore prepared:

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1. 25 c.c. KCl $2\frac{1}{2}m$ + 75 c.c. sea-water.
2. $22\frac{1}{2}$ c.c. KCl $2\frac{1}{2}m$ + $77\frac{1}{2}$ c.c. sea-water.
3. 20 c.c. KCl $2\frac{1}{2}m$ + 80 c.c. sea-water.
4. $17\frac{1}{2}$ c.c. KCl $2\frac{1}{2}m$ + $82\frac{1}{2}$ c.c. sea-water.
5. 15 c.c. KCl $2\frac{1}{2}m$ + 85 c.c. sea-water.
6. $12\frac{1}{2}$ c.c. KCl $2\frac{1}{2}m$ + $82\frac{1}{2}$ c.c. sea-water.
7. 10 c.c. KCl $2\frac{1}{2}m$ + 90 c.c. sea-water.
8. $7\frac{1}{2}$ c.c. KCl $2\frac{1}{2}m$ + $92\frac{1}{2}$ c.c. sea-water.
9. 5 c.c. KCl $2\frac{1}{2}m$ + 95 c.c. sea-water.
10. $2\frac{1}{2}$ c.c. KCl $2\frac{1}{2}m$ + $97\frac{1}{2}$ c.c. sea-water.
11. $17\frac{1}{2}$ c.c. NaCl $2\frac{1}{2}m$ + $82\frac{1}{2}$ c.c. sea-water.
12. Sterile sea-water (control).

Lot I was returned to sea-water after 45 minutes; Lot II, after 75, and Lot III, after 105 minutes.

To avoid repetition, I will only state in which solutions swimming larvæ were obtained, leaving a description of the histological findings until later. At 4.00 P. M. the first ciliated larvæ were discovered in Lot II of Solution 4. At 11.30 P. M., when it was certain that all the larvæ which would develop were swimming, conditions were as follows:

The majority of the eggs in the control were somewhat shrunken and opaque, and many had lost the peripheral arrangement of the oil globules, and were segmented into two and four cells. None were swimming.

All three lots of the NaCl-sea-water mixture were full of swimming larvæ. Many swimming larvæ developed in Lots I and II of Solutions 4 and 5. Several ciliated larvæ were also found in Lot II of Solutions 3, 6, and 7. The eggs from Solutions 1 and 2 were shrunken and granular. The capsules were swollen and the eggs were going to pieces. In solutions 8, 9, and 10, the majority of eggs had segmented into two and four cells, but none were swimming.

At noon on the following day, no swimming larvæ had developed in the control; a large number of the eggs were dying. Lots II and III of Solutions 4, 5, and 6 still contained many swimming larvæ. The eggs which had been in the sodium-chloride solution were still swimming beautifully. An occasional ciliated larva was found in Solution 7. In the other dishes everything was dead.

The experiment again showed that when the unfertilized eggs of Nereis are left for a certain time in sea-water, the concentration of which has been increased to a definite degree by the addition of either KCl or NaCl, they will develop into swimming larvæ when returned to ordinary sea-water.

*Experiment IV, July 10, 1902.* — 2.15 A. M. In the experiments described thus far, only electrolytes have been used to increase the concentration of the sea-water. If parthenogenesis in Nereis is due simply to the ab-

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straction of water from the egg, we should be able to bring about the development of the unfertilized egg, not only by mixtures of sea-water and electrolytes, but also by mixtures of sea-water and non-electrolytes. To test this point, the following solutions were prepared:

1. 50 c.c. cane-sugar 2 *m* + 50 c.c. sea-water.
2. 40 c.c. cane-sugar 2 *m* + 60 c.c. sea-water.
3. 30 c.c. cane-sugar 2 *m* + 70 c.c. sea-water.
4. 10 c.c. cane-sugar 2 *m* + 90 c.c. sea-water.
5. Sterile sea-water (control).

The first lot of eggs was removed from the solutions after 90 minutes, the second, after 110 minutes.

At 12.30 P.M., all the eggs which had been in Solutions 1 and 2 were dead. Both lots of eggs from Solution 3 were full of swimming larvæ. In the remaining dishes, every egg was intact.

A mixture of 30 c.c. cane-sugar 2 *m* + 70 c.c. sea-water has about the same osmotic pressure as a mixture of 15 c.c. KCl $2\frac{1}{2}$ *m* + 85 c.c. sea-water. It seems, therefore, as though the essential factor in bringing about artificial parthenogenesis in Nereis is an abstraction of water from the egg, and that it does not matter decidedly whether the concentration of the sea-water used for this purpose is raised by the addition of electrolytes or by the addition of non-electrolytes.

The above experiments were repeated several times, but always with the same results. The eggs of Nereis are not naturally parthenogenetic, but cleave and develop to the swimming stage if immersed for from one-half to one and one-half hours in sea-water, the concentration of which has been raised a definite amount, and then returned to ordinary sea-water. It does not matter whether electrolytes or non-electrolytes are used for this purpose, — sodium chloride, potassium chloride, cane-sugar may be used, — though sodium chloride usually yields the largest number of swimming larvæ, while sugar yields the least.

A series of experiments had been begun in which I attempted to obtain swimming larvæ by altering the ion concentration of the sea-water without altering its osmotic pressure, when the material gave out, and put an end to further work. Thus far the experiments in this direction have yielded only negative results; but they will be continued next year.

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THE DIFFERENCES BETWEEN THE NORMALLY FERTILIZED, PARTHENOGENETIC AND UNFERTILIZED EGGS OF NEREIS LIMBATA.

At ordinary summer temperature, the fertilized egg of Nereis limbata throws out its polar bodies about one hour after fertilization, and cleaves for the first time some fifteen or twenty minutes later. The second cleavage occurs about one hour and forty-five minutes after fertilization. Cleavage then proceeds in a regular manner, and the blastula stage is reached in seven hours; the eggs swim in about eleven hours, varying somewhat with the temperature.

When the unfertilized eggs are brought to the swimming stage through a temporary residence in sea-water, the osmotic concentration of which has been increased a definite amount, no special changes, save a slight shrinkage of the protoplasm, a disappearance of the nuclear membrane, and a slight increase in opacity, are noted while the eggs remain in the concentrated sea-water. An hour after being returned to ordinary sea-water, the egg protoplasm becomes more

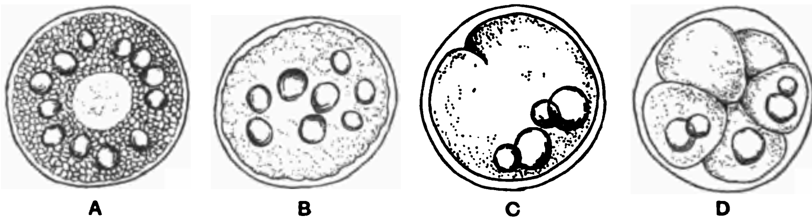


FIGURE 1. — *A*. Unsegmented egg of Nereis limbata immediately after its return to ordinary sea-water after immersion for seventy-five minutes in a mixture of 17½ c.c. 2½ *m* NaCl + 82½ c.c. sea-water. The eggs were returned to sea-water at 3 25 A. M. Experiment 3. *B*. Same egg, one hour later. The increase in the opacity of the protoplasm is not indicated in the drawing. *C*. Same egg, 4.55 A. M. *D*. Same egg, 5.10 A. M.

opaque and its outline somewhat irregular. The characteristic peripheral arrangement of the oil globules also disappears, and they become clumped irregularly in the central portions of the egg (Fig. 1, B). During the second hour after their return to ordinary sea-water, many of the eggs cleave into two cells, after which cleavage into four, eight, and sixteen cells may occur in a more or less regular manner (Fig. 1, C, D). Much oftener, however, no change whatever takes place in the two hours following the return of the eggs into normal sea-water, and then the eggs suddenly fissure irregularly, and break

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up at once into a number of spherules (cells ?) (Fig. 2, B, C). Cleavage may then continue until such pictures as are shown in Figures 3, A, B, C, D, E, are obtained which were made when the first evidences

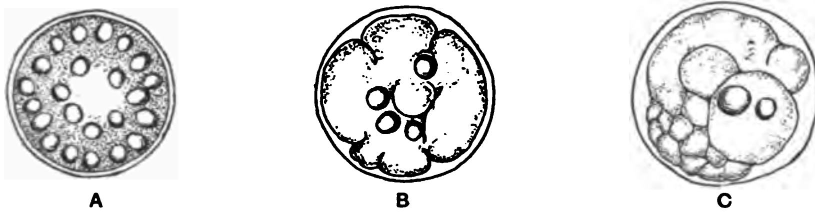


FIGURE 2. — *A*. Control egg from Experiment 3. *B*, *C*. Eggs which had been in a solution of 90 c.c. sea-water + 10 c.c. KCl $2\frac{1}{2}$ *m* for seventy-five minutes, and were then returned to sea-water. The eggs were removed from the solution at 3.25 A. M. The drawings were made at 6.00 A.M.

of ciliary movement were noticeable among the eggs. This stage is reached in about fourteen hours, though the majority of the eggs do not swim until six or ten hours later. The swimming stage is therefore reached at a much later time than when the eggs are fertilized

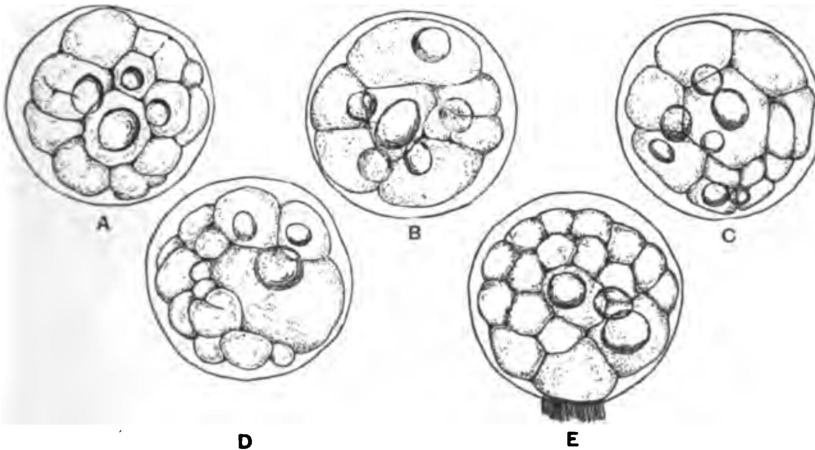


FIGURE 3. — Parthenogenetic larvæ from Experiment 2. The drawings were made fourteen hours after the return of the eggs from Solution 5 to ordinary sea-water. Some of the eggs were just beginning to swim.

by a spermatozoon. At other times, cleavage occurs in a much more regular manner (Fig. 4, A, C), and the swimming larvæ have an appearance which cannot be distinguished from that of trochophores

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produced through fertilization with sperm. It often occurs that lines of cleavage disappear, and cells coalesce, as Loeb found to be the case in Chætopterus.[1]

The unfertilized eggs of Nereis, when left in ordinary sea-water,

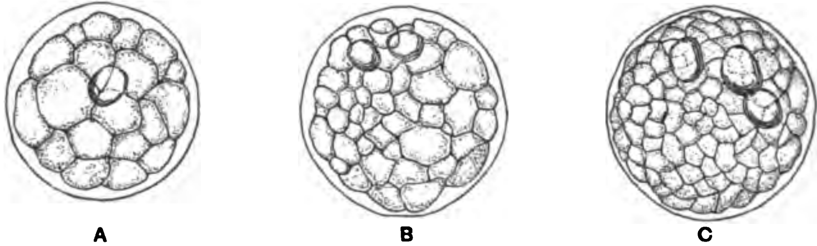


FIGURE 4. — Parthenogenetic larvæ from Solution 11 of Experiment 3. The eggs were swimming, but the cilia are not indicated in the drawings.

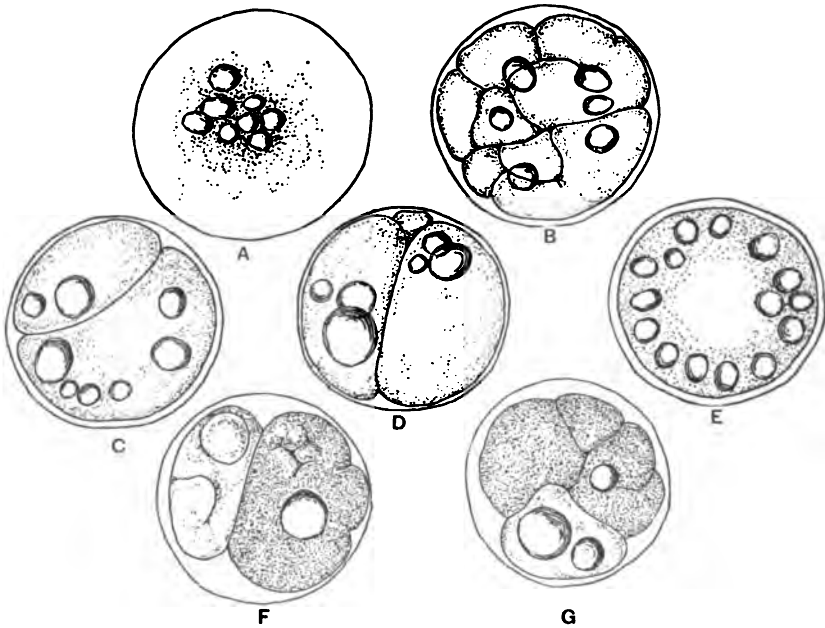


FIGURE 5. — Unfertilized control eggs of Nereis, showing the various changes they suffer when left in ordinary sea-water. *A*, *B*. Control eggs twenty-eight hours after their removal from the ovaries. The eggs cleaved, and then underwent a granular degeneration. *C*. Control egg from the same dish, practically intact. *D*, *E*, *F*. Control eggs in which cleavage occurred. *G*. A control egg in a state of granular disintegration.

[1] LOEB, J.: This journal, 1901, iv, p. 423.

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usually show no change during the first eight or twelve hours after their removal from the ovaries. Often they are absolutely unaltered after remaining in sea-water for thirty-six hours (Fig. 5, C), when they become opaque and granular and suddenly break up into a granular débris (Fig. 5, G). At other times the unfertilized eggs become slightly opaque after lying in sea-water for several hours, lose the peripheral arrangement of the oil globules, and go through one or two cleavages (Fig. 5, D, E). They then become granular and go to pieces (Fig. 5, A, B). Some of the eggs may even divide into a dozen cells (Fig. 5, F). Never, however, do the unfertilized eggs develop into swimming larvæ. It can often be seen that while one dish of control eggs will show no evidences of cleavage whatsoever, another dish of the same eggs will show a large number in the two or four cell stage. Mere mechanical agitation is certainly not responsible for this change, for I have often found that while none of the eggs which had been transferred from one dish to another showed any signs of development, those which had been kept absolutely undisturbed showed a large number of eggs in the two or four cell stage. In looking over the notes of my experiments, I find that such evidences of development occurred most often in dishes containing large numbers of eggs. It is possible, therefore, that lack of oxygen or the formation of carbon dioxide lie at the basis of the process, — a question which will be studied next summer.

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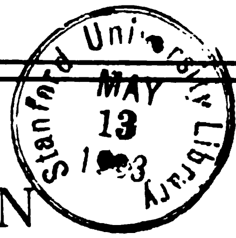


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# THE IMMUNITY OF FUNDULUS EGGS AND EMBRYOS TO ELECTRICAL STIMULATION.

By ORVILLE H. BROWN.

[*From the Hull Physiological Laboratories of the University of Chicago.*]

IT has been shown by Loeb[1] that the eggs and the embryos of the small fish Fundulus heteroclytus are peculiarly resistant to sudden changes of osmotic pressure of the media in which they live. The eggs when fertilized will develop and live in distilled water, in sea-water, or even in concentrated sea-water. The most probable explanation of this curious fact seemed to be that the tissues were as easily permeable to salts in the water as to the water itself. If this is true, polarization will not easily occur in such eggs when subjected to an electrical current; and if the stimulating action of an electrical current is due primarily to polarization, the egg and the young fish should be almost immune to such a current. Dr. A. P. Mathews suggested that I compare the effect of the current on the egg, the embryo, and the adult fish. The results confirmed our expectations. It was found that the eggs are polarized with great difficulty; the embryos show almost a complete immunity to electrical stimulation, while the adult fish reacts to even a weak current in a positive manner.

## Methods.

It was necessary to observe the eggs under a microscope. To make this possible, a small paraffin cell was moulded upon an ordinary glass slip. Into each end of the cell was inserted the tip of one of Porter's non-polarizable boot electrodes. The boot electrodes were attached to Mesco dry batteries, and also to Daniell elements, which were used in connection with a voltmeter. The Fundulus eggs were surrounded in different experiments with water the density of which varied from sea-water to distilled water. In some cases most of the water was removed and the eggs were placed in contact from one electrode to the other. Control experiments were performed upon

[1] Loeb, J.: Archiv für die gesammte Physiologie, 1894, lv, p. 390.



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Asterias and Arbacia eggs. As these Echinoderm eggs go to pieces in distilled water, they were placed in a normal solution of urea. It was necessary to place the eggs in solutions which were very poor conductors, so as to cause the passage of the maximum amount of current through the eggs. Exact comparisons of the effect of the current on the Fundulus, and Asterias and Arbacia eggs cannot be made, since the retaining solutions did not have the same power to conduct the current. The free swimming embryos and the adult fishes were worked upon in a rectangular dish, the dimensions of which were two and a half inches by five inches. The dish contained only sufficient distilled or other water to allow free swimming movements. The current was conducted into the water in these cases by means of large electrodes at the ends of the dish.

**A. Effects of the current on the eggs.** *Experiment I.* — It had been ascertained by previous experiment that currents of ordinary strength had no apparent effect upon either the Fundulus eggs or the embryos. The eggs were placed in distilled water. The strength of the current was increased to fifteen cells with but little effect; eighteen cells produced definite results. To make sure that the current was good in every case, the cells and the circuit were tested by the voltmeter. The protoplasm retracted from the side near the negative electrode, and travelled toward the opposite side, where liquefaction of the membrane occurred. Some of the yolk of the egg and many of the oil globules escaped and travelled with the cathode stream, confirming Lillie's[1] results. This required a constant passage of the current for fifteen to twenty minutes. With this strength of current, however, many of the eggs did not burst and allow an escape of the egg substance. There was only a shrinking of the inner membrane and the yolk of the egg.

*Experiment II.* — This was performed upon Asterias and Arbacia eggs in normal urea solution, as a control experiment. Ten dry cells were used. These were sufficient to cause a great many of the eggs to liquefy and burst upon the side near the positive electrode, and allow an escape of the egg substance (*vide* Lillie).

*Experiment III.* — The object of this experiment was to see whether the developing Fundulus eggs are affected by the passage of a current of medium strength. The eggs were fertilized, and when in the two-cell stage, they were subjected in distilled water to the current of five cells, which was allowed to flow for an hour, at the end of which time the eggs had developed to the sixty-four-cell stage, apparently with no irregularities.

[1] LILLIE, R. S.: This journal, 1903, viii, p. 273.

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**B. Effect of the current on the embryos.** *Experiment I.* — Four day old embryos, the hearts of which were just beginning to beat, were placed in the paraffin cell containing distilled water through which the current from twenty-one cells was passing. There was no twitch at either make or break. The heart maintained apparently the same rhythm as before the passage of the current.

*Experiment II.* — Embryos twenty-one days old were placed in the rectangular dish containing a shallow layer of distilled water. Nothing less than eighteen cells would cause a twitching of the small fish. By the use of twenty-one cells, they responded somewhat as the adult responds to a very much weaker current, as will be shown. When the fish were in sea-water, or fresh water, the current had no effect.

**C. Effect of the current on the adult.** *Experiment I.* — The normal adult Fundulus was placed in the same dish as was used in the previous experiment. There was just sufficient fresh water in the dish to cover the fish completely. By the use of one cell the fish responded to the make shock with a jump. It turned toward the anode and swam to it. By the use of three cells, the following results were obtained:

1. If the fish was turned with its head toward the anode when the current was closed, it was paralyzed and laid over on its side absolutely relaxed. Breaking the current caused Ritter's tetanus.
2. If the fish was turned with its head toward the cathode when the circuit was completed, it immediately turned around until its head was toward the anode, and turned over onto one side, paralyzed.
3. If the fish was held in the stream with its head toward the anode, it remained quiet.
4. If held with its head toward the cathode, it endeavored to turn. The fins were in rapid vibration, and the tips of the pectoral fins pointed toward the cathode.
5. When the fish was held by its head crosswise of the current, its tail turned toward the anode. The fin on the anode side was held at an angle of about forty-five degrees from the part of the body posterior to the fin, while the fin on the cathode side was held nearly at right angles to the same part of the body.
6. When a fish was held by the middle of the body, crosswise of the current, its head was turned toward the anode.

*Experiment II.* — This experiment was to show the effect of the current on the fish after the brain has been destroyed. The method was the same as in the experiment just preceding.

1. If its head was toward the anode, using four cells when the circuit was closed, the animal made a feeble jump toward the anode and became quiet.

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2. If its head was toward the cathode at the make of the current, the fish made efforts to turn away from the cathode, but it never succeeded.
3. The same response to the current that Loeb and Garrey[1] observed in the larvæ of Amblystoma, was also detected in the adult Fundulus with the brain destroyed. That is, when the current flows from the head to the tail, the back becomes arched, while if the current passes from the tail to the head, the back becomes concave, both the head and the tail being raised.
4. When the animal is crosswise of the current, the fins move at make or break of the current, but neither the head nor the tail turn toward the anode as they did in the normal animal.

*Experiment III.* — The object of this experiment was to show the effect of the current upon the fish when both the brain and the spinal cord are destroyed. The method was the same as in the two preceding experiments. The same strength of current was employed. All the responses were very slight.

1. When the fish was held on its ventral side, with its head toward the anode, no responses other than a slight movement of the fins at make or break were made.
2. When its head was toward the cathode, the pectoral fins were held nearly at right angles from the body, with their tips slightly curved toward the cathode.
3. When the animal was turned at right angles to the current, a twitch of the fins was noticed, which was about as great on one side as the other.
4. The same phenomena as was observed in the animal with only the brain destroyed, were also seen here, *i.e.*, the descending current caused the body to be arched, while the ascending current caused the head and tail to be raised.

## Summary and Conclusions.

1. The fact that the eggs of Fundulus are immune to electrical currents, as they are to osmotic changes of the medium in which they live, is an interesting physiological confirmation of the theory of the osmotic nature of electrolysis. The most probable explanation appears to be that the membranes of the egg are so freely permeable to ions and possibly to neutral particles that no polarization can occur.

2. This conclusion is supported by the behavior of Arbacia and Asterias eggs, which show the contrary relation, being readily susceptible both to electric currents and to osmotic changes.

[1] Loeb and Garrey: Archiv für die gesammte Physiologie, 1896, lxv, p. 41.

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3. The fact that these eggs and embryos are not affected by a current, except when very strong, supports the hypothesis that electrical stimulation depends primarily upon polarization where the current enters and leaves the cell.

4. There is a gradual increase in susceptibility to osmotic changes and to the electric current as the embryo develops, the adult fish being readily stimulated by the current from a single cell, which is quite without action in the embryo.

5. It is suggested that the resistance of the electric and other fishes to electric stimulation, noted by Du Bois-Reymond and others, may not improbably be similarly explained by the permeability of the walls of their tissue cells to ions.

6. The liquefaction of the eggs on the anode side, and the quieting effect of the anode, supports Mathews' hypothesis of the dissolving action of the cathions and their inhibitory action.

7. The galvanotropic reactions are shown to depend primarily upon the nervous system, as already made out by Loeb in other forms.

I take this opportunity to thank Dr. A. P. Mathews and Dr. R. S. Lillie for suggestions and aid.

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# ON THE INFLUENCE OF VARYING INTENSITIES AND QUALITIES OF VISUAL STIMULATION UPON THE RAPIDITY OF REACTIONS TO AUDITORY STIMULI.

By ROBERT MacDOUGALL.

THE interaction of hetero-sensorial stimulations and the influence of sensory stimuli upon simultaneously existing motor processes have been reported for a variety of activities. Such, for example, is the influence of musical tones in sharpening the perception of color when the stimulus is below the threshold of unassisted vision; such is also the reinforcement or reduction of force which a maximum motor reaction receives from incident visual stimulations of specific qualities. It is but a step to extend the application of the concept to other sensori-motor relations. In the present paper is reported a preliminary investigation concerning one of these aspects.

Reaction times — the period required to make a movement in response to a preconcerted signal — have been found to vary in dependence upon at least the following factors: *a*, the sensory type of signal employed, *b*, its intensity, *c*, its location, *d*, the presence or absence of a preceding warning, *e*, the period intervening between warning and stimulus, *f*, the nature of the reaction which is made, and *g*, its energy. The question here raised is whether, in addition to these special factors of variation, the intensity and quality of the general stimulation falling upon the organism affect the rapidity of its responses in a way analogous to the dynamogenic influence upon reactions of these stimuli.

Two factors of possible variation were studied: first, changes in the intensity of the stimulation falling upon the retina, reactions being made: *a*, in darkness, *b*, in weak light, and *c*, in strong light; and second, changes in the quality of the visual stimulation, reactions being made under illumination by the following homogeneous lights: *a*, red, *b*, orange, *c*, green, and *d*, blue.

The subject sat before a table with his right forefinger on the re-



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action key. His head was enclosed in a blackened box having a front of milk-glass, by which the distracting influence of a diversified field of view was eliminated. The variations in light intensity were controlled by the use of sixteen and two candle-power electric bulbs, and by shutting off the illumination completely. The reaction stimulus consisted of the click of a telephone receiver attached to the head over the ear. A preparatory signal — also auditory — was given two seconds before the application of the stimulus. The reactor directed his attention to the response to be made, as this attitude appears most conducive to regularity, as well as rapidity in the reactions. Records were made by a Hipp chronoscope, corrected daily by means of a gravity chronoscope. Preliminary training was given in a series of experiments to establish the normal reaction times of the subjects in ordinary daylight. Two persons took part in the experiment.[1] In view of this fact and the circumstance that only five series of ten reactions each were ordinarily used in making up the individual averages, the quantitative results presented must be held subject to revision.

The reaction times obtained by varying the intensity of the incident light are exhibited in the following table:

TABLE I.

| Subject. | Darkness. | 2 c.p. | 16 c.p. |
|---|---|---|---|
| A. | 140.6 $\sigma$ | 145.7 $\sigma$ | 152.7 $\sigma$ |
| B. | 139.7 $\sigma$ | 137.3 $\sigma$ | 144.2 $\sigma$ |
| Average | 140.1 $\sigma$ | 141.5 $\sigma$ | 148.4 $\sigma$ |

No dynamic relation appears between intensity in the illumination and rapidity in the response. The reaction time is shorter in darkness than in light in the ratio — including both intensities — of 140.1 to 144.9. The reaction time is also shorter under the weaker than under the stronger illumination, the difference being more marked between weak and strong light than between illumination and darkness.

[1] Messrs. W. E. Hocking and W. P. Burris, to whom I am indebted for carrying out the whole series of reactions.

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The mean variations within the reaction series are given in the following table:

TABLE II.

| Subject. | Darkness. | 2 c.p. | 16 c.p. |
|---|---|---|---|
| A. | 6.4% | 7.1% | 6.5% |
| B. | 10.7% | 11.5% | 7.4% |
| Average. | 8.5% | 9.3% | 6.9% |

The regularity of the responses under these conditions is not noticeably affected by the change from light to darkness, the mean variations, being, respectively, 8.1 per cent and 8.5 per cent. Both reactors show an increase in inconstancy in passing from strong to weak light, — 6.9 per cent to 9.3 per cent, — the latter showing also more disturbance than darkness. The responses are thus swifter and more irregular in faint light and in darkness than under conditions of illumination more nearly approximating ordinary daylight, results which suggest the influence of changing mental attitudes due to factors of novelty and attention, rather than any direct dynamogenic relation between sensory and motor processes.

The reaction times obtained under homogeneous light are given in the following table:

TABLE III.

| Subject. | Red. | Orange. | Green. | Blue. |
|---|---|---|---|---|
| A. | 134.1 σ | 123.1 σ | 129.2 σ | 130.2 σ |
| B. | 125.1 σ | 139.2 σ | 132.2 σ | 132.9 σ |
| Average. | 129.6 σ | 131.1 σ | 130.7 σ | 131.5 σ |

The reactions under colored light are made more rapidly than under neutral light, in the ratio of 130.7 to 148.4. This acceleration is presented by both reactors under every quality of homogeneous light. The reactions made under colored light are shorter also than those made in darkness, in the ratio of 130.7 to 140.0. No constant relation appears between the various color qualities and the time of reaction. Extreme rates, in the case of both

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subjects, occur under red and orange stimulation, intermediate rates with blue and green. The reaction times of the two subjects follow the same curves in blue and green light, but converse curves in red and orange, and the differences are in all cases slight.

The regularity of the responses is greater under neutral than colored light, in the ratio of 6.9 per cent to 7.6 per cent. The values of the mean variation under the latter conditions are given in the following table:

TABLE IV.

| Subject. | Red. | Orange. | Green. | Blue. |
|---|---|---|---|---|
| A. | 7.8% | 6.7% | 4.9% | 7.3% |
| B. | 7.0% | 10.4% | 8.7% | 8.1% |
| Average. | 7.4% | 8.5% | 6.8% | 7.7% |

The responses are thus swifter and more irregular under colored than neutral illumination, but, as in the latter case, no direct dynamic relation appears between sensory and motor processes.

The differences which do appear point rather to a waxing and waning in the interest with which the reactor attends to a stimulus under conditions of relative novelty and familiarity respectively, and to a facilitation of the nervous discharges in general. When the reaction series were arranged in pairs, and a change in the quality of the light was made in passing from the first to the second set, the reaction time in the later series was distinctly reduced. With each reactor this change appeared in five out of the six color successions introduced, as shown in the following table:

TABLE V.

| Succession of colors. | Subject A. | | Subject B. | |
|---|---|---|---|---|
| | Series 1. | Series 2. | Series 1. | Series 2. |
| Red followed by blue . . . | 160 σ | 151 σ | 167 σ | 163 σ |
| Blue followed by red . . . | 159 σ | 147 σ | 168 σ | 162 σ |
| Blue followed by orange . . | 151 σ | 148 σ | 154 σ | 146 σ |
| Orange followed by blue . . | 152 σ | 142 σ | 150 σ | 162 σ |
| Green followed by blue . . | 136 σ | 152 σ | 165 σ | 149 σ |

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Such a shortening of the reaction time takes place as the result of simple habituation, and the effects of this process might be expected to appear within the period under discussion. That the phenomenon is not a practice effect is shown by the results of introducing paired series in which the reactions in each of the successive sets were made under the same conditions of stimulation. Reaction times are presented in regard to three colors only, two of which are partial, since the repetition of color series was not made for the purpose of determining the present point, and its occurrence is, in this connection, accidental.

TABLE VI.

| Colors. | Subject A. | | Subject B. | |
|---|---|---|---|---|
| | Series 1. | Series 2. | Series 1. | Series 2. |
| Red . . . | 162 σ | 167 σ | 151 σ | 159 σ |
| Orange . . | .... | .... | 141 σ | 152 σ |
| Blue . . . | 146 σ | 154 σ | | |

The second series, in each of these four cases, exhibits an increase in the reaction time, instead of the decrease which is to be looked for as the result of practice. The only discernible difference in the two cases lies in the relation of the reaction to its sensory environment, which in the one instance presented the maintenance of the preceding conditions of general stimulation, and in the other involved a passage to circumstances possessing elements of relative novelty. It is possible that colored light exerts a dynamogenic influence upon reaction, and that its effects die away as the reactor becomes habituated to the stimulation. It seems more reasonable, however, to attribute the phenomenon to fluctuations in the general alertness of the reactor, which take place in dependence upon changes in his surroundings. The attitude of attention is characterized by nervous facilitation, and an increase in the general bodily innervations, tending to produce both an acceleration in the responses made to stimulation, and an intensification of their force. This appears in the relation which has been found to hold between the reaction time and the force with which the movement is executed. It makes it probable also, if means were devised for measuring the intensity of each reaction as it took

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place, that the correlation between force and swiftness in the response might account for the differences which exist between so-called sensory and motor reaction times. It is in this relation, further, that we should look for a partial explanation of the acceleration which follows the use of a signal preparatory to the application of the stimulus, for I am convinced that if the force of the reactions were recorded in the two cases, it would be found that a higher general average would be presented when a warning is employed, than when the stimulus is applied without a preparatory signal. In short, the factor determining the rate of response to homogeneous sensory stimuli appears not to be material differences in their quality or intensity, but the degree to which they succeed in stimulating the attention of the reactor, and thereby increasing the general nervous excitability of the moment.

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# ON THE RELATION OF EYE MOVEMENTS TO LIMITING VISUAL STIMULI.

By ROBERT MacDOUGALL.

THE general phenomena of geometrical optical illusions point to a constant relation between the movements of the eye and the varying intensities and qualities of luminous objects within the field of vision. Reflex movements are incessantly initiated by the appearance of strong local stimuli, and voluntary movements, with scarcely less frequency, are affected by the presence of unequal intensities and qualities of illumination in different parts of the field of view, as the point of regard moves over it. Theoretically, it may be said that every bright point or object one sees, either causes a movement of the eye, or inhibits it if it is in progress, — including within the term any retardation as well as the complete cessation of motion. These unregarded factors, causing movement where none is intended or recognized, and transforming it in greater or less degree when it is in process, give rise to a variety of errors in our judgments of distance, direction, size, form, and the like.

The investigation here reported, which concerns one of these types of distorted judgments, took shape from the results of certain observations made in connection with an experimental study of the location of the subjective horizon.[1] Judgments were made in a darkened room, by means of an illuminated disc, running upon a vertical carriage, placed at some two metres distance from the observer, who, by means of a system of cords, adjusted the disc upon the imaginary horizon. At the top and bottom of this carriage, electric bulbs were placed, by which, at the pressure of a button, a small rectangle could be illuminated with diffused neutral light. The purpose of these arrangements was not made known to the observer, who was directed to adjust the disc without reference to them. The results of one hundred and

[1] Published in Harvard Psychological Studies, Vol. I. (Monograph Supplement 17, of the Psychological Review).



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ninety observations, distributed among six subjects, showed a difference in the location of the imaginary horizon under the two conditions of 32.71′ of arc. In the article referred to, the following comments are made: "The eye is uniformly attracted toward the light, and the location of the disc correspondingly elevated or depressed. The amount of displacement which appears is relatively large. It will be found to vary with the intensity, extent, and distance of the illuminated surfaces introduced. There can be little doubt that the practical judgments of life are likewise affected by the distribution of light intensities, and possibly also of significant objects, above and below the horizon belt. Every brilliant object attracts the eye toward itself; and the horizon beneath a low sun or moon will be found to be located higher than in a clouded sky."

In order to examine certain of these factors in more detail, a series of experiments was arranged in which the observer was called upon to locate the median point between two limits disposed in a horizontal line, one of which remained constant, while the other was varied in size, form, brightness, and color, successively.

A vertical black screen, eighty centimetres square, was set up at a distance of two metres from the observer. Two rectangles of white cardboard, five by twenty millimetres in size, and fifty centimetres apart on a horizontal line, were attached to this screen with their longer axes vertical. These formed the limits during the preliminary series of determinations in which the normal variability was established for each observer. A third card, similar to these limits, was attached to a fine black cord passing over pulleys at the sides of the screen, and thence to the observer, whose judgments were directly recorded by adjusting this travelling index at what appeared to be the middle point between the two extremes, the reckoning being always from the inner boundaries of the limiting areas, whatever the character of these might be. The observer then sat with closed eyes while his location of the median point was recorded, and the index displaced, — now to the right, then to the left, — in preparation for the following experiment. Records were made in terms of displacement from the objective centre, those toward the variable limits being marked (+), those toward the constant limit (−). Twenty judgments were taken with the variable limit on the right, and a similar number with it on the left, in each case ten judgments being made after displacement of the index to the one hand, and ten after displacement to the other. Forty judgments thus formed the basis of each individual average, while three observers

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took part in the experiments, giving a total of one hundred and twenty reactions for each set of figures presented in the results.[1]

Six series of changes were made in the variable limit, as follows: The constant limit was replaced, 1, by vertical strips of white cardboard two centimetres in width, and successively two, four, eight, twelve, sixteen, and twenty centimetres in length; 2, by similar horizontal strips; 3, by squares of two, four, and six centimetre sides; 4, by two strips inclined to form an angle, which were, *a*, three centimetres in length and enclosed angles of forty, ninety, and one hundred and forty degrees, respectively, and, *b*, six centimetres in length, enclosing the same angles; 5, by areas equal to the constant limit, but of light gray, dark gray, and black cardboard respectively; and, 6, by areas equal to the constant limit, but of the following colors: red, orange, yellow, green, blue, and violet.

The quantitative results of locations made under the first two series of changes are given in the following tables:

TABLE I.

VARIABLE LIMIT HORIZONTALLY EXTENDED.

| Length. | 4 cm. | 8 cm. | 12 cm. | 16 cm. | 20 cm. |
|---|---|---|---|---|---|
| A.D. | 3.27 | 2.90 | 2.83 | 3.13 | 3.98 |
| C.E. | +2.38 | +0.93 | −0.15 | −1.46 | −2.48 |
| M.V. | 2.08 | 2.12 | 2.90 | 3.08 | 2.96 |

TABLE II.

VARIABLE LIMIT VERTICALLY EXTENDED.

| Length. | 4 cm. | 8 cm. | 12 cm. | 16 cm. | 20 cm. |
|---|---|---|---|---|---|
| A.D. | 2.55 | 2.76 | 3.26 | 3.47 | 3.38 |
| C.E. | −0.56 | −0.71 | −0.25 | +0.93 | +0.73 |
| M.V. | 2.58 | 2.55 | 2.98 | 2.56 | 2.73 |

Throughout the tables A.D. stands for the average deviation of the locations from the objective centre; C.E. for the constant error involved in the series of judgments; and M.V. for the mean variation of the same series. The measurements are recorded in millimetres.

[1] I am indebted to Messrs. BREWER, CAREY, and MEAKIN for the record of udgments upon which this paper is based.

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Vertical elongation of the variable limit is followed by no noticeable variation in the judgments; neither constant error nor mean variation shows concomitant changes. The average deviation alone increases progressively. Horizontal extension of the variable limit is followed by a deflection of judgment toward it only at the beginning of its enlargement, and in more than half of the series the error which appears is negative in sign. In the series of horizontal changes the mean variation increases with the elongation of the strip, except in the case of the last increment, where an inversion of the curve takes place. In the series of vertical changes no such increase is manifested. The eye movements which the process of judgment involves are made in a horizontal line, along which axis, in the latter case, the limiting stimuli do not vary; in the horizontal series, on the contrary, the limit changes continually in the line of the eye movements, and in such a way as to offer an enlarging range of possible variations in the eye movements made, and therefore a ground for the progressive increase in the irregularity which is manifested in the mean variation.

The results of substituting a series of increasing squares for one of the original limits are given in the following tables:

TABLE III.

VARIABLE LIMIT A SERIES OF SQUARES.

| Length. | 2 cm. | 4 cm. | 6 cm. |
|---|---|---|---|
| A.D. | 2.77 | 3.48 | 2.85 |
| C.E. | +0.55 | +1.78 | +1.03 |
| M.V. | 2.08 | 2.35 | 1.96 |

The influence of this series of changes also is relatively slight. The location of the median point is deflected toward the variable limit, the amount of error at first increasing with the enlargement of the square but falling again when the latter is much larger than the constant limit. Average deviation and mean variation follow similar curves, appearing as constant functions of the deflection of judgment. The form of these curves appears to be due to a change in the mode in which the variable limit enters into the process of estimation. It is probable that in the case of the smaller squares the whole area is regarded as a unit, and the extent of the eye movements, together with their variability, increases with each remove of the centre of the

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figure from the opposite limit; but that when a certain magnitude has been reached the square is no longer regarded as a whole, but only the nearer border of it taken into account, in consequence of which there results a decrease in the total excursions of the eye, and an increase in the regularity of the judgments consequent upon the greater definition of the termini between which the movements take place.

More constant and marked effects follow the introduction of the fourth series of changes. Here the variable limit consisted of a piece of white cardboard, cut so as to form an angle having its apex toward the opposite limit, the sides composing the figure being five millimetres in width and of either of two lengths, thirty millimetres in the first sub-group and sixty in the second. In each set the enclosed angle was made successively of 140, 90, and 40 degrees magnitude. The results are given in the following tables:

TABLE IV.

SIDES 30 MM. LONG.

| Angle. | 140° | 90° | 40° |
|---|---|---|---|
| A.D. | 2.43 | 3.45 | 4.80 |
| C.E. | +0.55 | +2.80 | +4.56 |
| M.V. | 1.94 | 2.12 | 2.00 |

TABLE V.

SIDES 60 MM. LONG.

| Angle. | 140° | 90° | 40° |
|---|---|---|---|
| A.D. | 3.48 | 5.25 | 5.68 |
| C.E. | +2.70 | +4.21 | +5.85 |
| M.V. | 2.55 | 2.36 | 2.88 |

The deflection in the location of the median point presented in these tables is positive in direction, large in amount, and varies concomitantly with the factors of change introduced. The constant error in both sub-groups varies inversely with the magnitude of the enclosed angle, the average deviation following a similar curve. The amount of deflection also varies directly with the length of the enclosing sides.

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The influence of the length of sides is least in the case of the smallest angles, and greatest in those of largest magnitude. The whole experimental series composes a quantitative estimation of the illusion in one form of the Müller-Lyer figure. The constant factor of change in both the above sets of conditions is the spatial disposition of the total figure which the varying angles and lines compose. The centre of this system of lines is removed farther from the opposite limit by each increase in length of the sides enclosing a constant angle, and by each reduction in the magnitude of an angle enclosed by lines of a constant length. With the variations in the position of this point, the amount of the positive errors in judgment rises and falls.

At the same time the reactions toward this changing figure are fairly definite and constant. The mean variation does not materially change with the alterations made in the character of the limit. The eye, in other words, apprehends the figure in each case as a structurally constant unit, and returns to a specific point of rest determined by the relations of the lines composing it.

The results of the last two series of changes, which may be discussed together, are presented in the following tables:

TABLE VI.

VARIABLE LIMIT: BRIGHTNESSES.

| Intensity. | Black. | Dark gray. | Light gray. |
|---|---|---|---|
| A.D. | 2.91 | 3.70 | 2.20 |
| C.E. | +1.45 | +0.73 | +0.42 |
| M.V. | 1.98 | 2.48 | 2 30 |

TABLE VII

VARIABLE LIMIT: COLORS.

| Color. | Red. | Orange. | Yellow. | Green. | Blue. | Violet. |
|---|---|---|---|---|---|---|
| A.D. | 2.93 | 2.02 | 2.63 | 3.38 | 3.58 | 3.57 |
| C.E. | +0.48 | +0.65 | −0.66 | +2.36 | +2.30 | +2.42 |
| M.V. | 2.43 | 1.85 | 2.35 | 2.38 | 2.36 | 2.07 |

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In the case of intensive variations the amount of deflection increases with each increment of difference between the constant and variable limits; and in each case the deflection is toward the stimulus which both is absolutely the weaker of the two and presents less contrast with the general background, from which relation also a stimulus to reflex movements of the eye might be conceived to arise. The source of this constant error must lie in some factor other than the sensational intensity of the limit, and to a common cause are apparently due the distortions which arise from both intensive and qualitative variations. In the latter case the following facts appear. If the spectrum be divided into two parts, red, orange, and yellow, or bright colors; and green, blue, and violet, or dark colors; the former group, approaching the white limit more nearly in brightness, have less disturbing effect upon the judgment than any of the latter group, and the deflections which occur are not toward the more intense stimulus, but toward the darker green, blue, and violet areas. The series of judgments is also more erratic when colors of the dark group are used than with those of the bright group. In general, then, the results parallel those which appear in connection with the series of brightnesses.

I am of opinion that these phenomena in general, namely, the factors of variability in the visual perception of magnitude and distance, are always to be explained in connection with the specific dynamogenic values of the whole system of elements which the field of view at any time contains. If illusions of direction appear, or deflections in the judgment of distances, they occur because the visual objects on the one side, or toward the one term, of the line of eye movement, exert a greater reflex attraction upon the eye, and make it more easy to turn it toward that side, and more difficult to turn it away than is the case with movements in the opposite direction. The primary dynamogenic quality of a luminous surface is its intensity; the brighter or more vivid an object, the more extended and uniform the reactions of rotation which it is capable of calling forth. This relation is exemplified in the distortion of judgment caused by the introduction of a bright light to the one side or other of a point to be located. It is a factor of recognized value in artistic composition, in which gold and color and brilliancy are applied to objects of secondary importance, —fabrics, decoration, foliage, skies, etc., — with the result that while attention is not specifically attracted toward them as individuals, these objects, nevertheless, decoy the eye to the centre of the system of

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motives incorporated in the picture, and thus preserve the artistic balance of the composition.

But here the natural series of values is inverted; the location of the half-way point is deflected toward the darkest gray and dullest colors, not toward the more stimulating white strip of paper. Other factors than that of intensity must be present. We should undoubtedly find that the whole series of elements which are taken into account in pictorial composition, have their place in the system of factors which determines such simple eye movements as those in question. Two of these factors appear to be chiefly at work in producing the variations presented by this set of experiments. The first of these, which characterizes the four geometrical series, is the habit of the eye, when exploring a system of visual objects in a limited field, to come to rest in a position at which the stimulations to reflex movements of rotation in opposite directions are in equilibrium. Such is the centre of a circle, or the intersection of the system of diameters of any symmetrical figure. This principle is most clearly exhibited in the phenomena presented by the introduction of the various angular forms, in which, as has been said, the changes which appear in the location of the median point will be found to parallel the shiftings of the centre of the figure defined by the apex of the angle and the terminations of its enclosing sides. It is, perhaps, too much to say that this centre is represented as the termination of the line whose centre is to be located; nevertheless the system of lines which compose this figure facilitate movements toward its centre, and retard those directed away from it more strongly than does the less striking patch of light which forms the opposite boundary of the distance to be bisected, and thereby introduces a characteristic distortion into the judgment.

The other factor to which reference has been made, is that of novelty, — an element less easily demonstrable than the preceding. The biological importance of habitual, prompt reaction upon novel stimuli is evident as are the power and constancy of the appeal which such stimuli actually make. To this ultilitarian value an element of intrinsic worth in the novel stimulus must be added; it is both more important that the novel object should be attended to, and more pleasant to receive the impression which it affords. These aspects are both independent of the primitive sensational intensity of the stimulus. In the series of brightnesses, and in that of colors as well, a disturbance appears which does not reflect any consistent qualitative or intensive change, but parallels the increments of difference

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between the variable form and the unchanging, habitual limit. Since these two sets of experiments took place at the close of the whole piece of work, we may fairly attribute to both colors and neutral shades an element of novelty, as compared with the constant white strip which limited the prescribed eye movements on the opposite side, and to this factor I ascribe the positive errors which are presented in the results.

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# ON THE IRRITABILITY OF THE BRAIN DURING ANÆMIA.

BY WILLIAM J. GIES.

[*From the Physiological Institute of Bern University.*]

## I. INTRODUCTION.

DURING the summer of 1899 I had the pleasure of assisting Professor Kronecker in a study of the irritability of the brain during anæmia.[1] Our research could not be concluded during my stay in Bern that summer, but we both looked forward to completing it together in the following year. Unfortunately for me, return to the Physiological Institute has been impossible thus far, and the work which has been delayed on that account has lately been resumed by Professor Kronecker and Dr. Stumme. At the suggestion of Professor Kronecker, the results of our investigation are presented here in some detail though briefly.

In the preparation of these notes I have received numerous suggestions from Professor Kronecker, who has also revised the statements relating directly to our experiments. Throughout practically all of our research, Professor Kronecker not only directed the work, but did a very large share of it. His well-known generosity to his pupils is again shown by his desire that this investigation, which was chiefly his, shall seem to be wholly mine.

## II. DESCRIPTION OF EXPERIMENTS.

In this research we sought especially to determine the order of cessation, as well as the period of continuance, of certain reflexes during anæmia of the brain.

Acute anæmia was brought about by perfusion with the solutions indicated on the next page.

The animals employed were toads, frogs, rabbits, and dogs.

[1] GIES: Report of the British Association for the Advancement of Science, 1899 (Dover), p. 897.



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The solutions used were various strengths of pure sodium chloride, Ringer's solution, and modifications of it, Schücking's solution (both of sodium and calcium saccharates), rabbit and horse serum, and 0.7 per cent sodium-chloride solution containing paraxanthin or chloralbacid.

**Experiments on toads and frogs.** — Perfusion in the cold-blooded animals was conducted with the least possible pressure through the abdominal vein. In this series of experiments we used all of the various solutions already enumerated, except serum.

Seventeen experiments were made, seven with toads and ten with frogs, each of which was continued for a period of from one to nine hours. The total amount of perfused fluid varied from 25 c.c. to 1590 c.c. In most cases perfusion was continued until the heart ceased to beat.

The table on page 133 gives a summary of the more important results obtained in this connection. The terms "skin," "lid," and "nose," in the table, refer to the reflex movements caused by pressure on those parts.

During the period of perfusion, the following functions gradually weakened, and then usually disappeared in this order: (1) respiration, (2) skin reflex, (3) lid reflex, (4) nose reflex, (5) heart beat.

The relative time of cessation of these reflexes varied considerably, not only with the character of the solutions, but also with the rapidity of their perfusion and the amounts used.

Convulsive extension of the limbs occurred in all the experiments in the earlier stages, but toward the close of each experiment and before the reflexive movements of the eyelids ceased, no such manifestations were observed, nor could they be induced by mechanical stimulation.

Perfusion of physiological saline solution containing 0.03 per cent of paraxanthin induced hyperæsthesia at first, but the reflex responses quickly came to an end, as the perfusion continued. Cumulative muscular rigor was the most pronounced feature of the experiment. At the end of the experiment the body was perfectly stiff. With a solution containing 0.015 per cent paraxanthin, moderate hyperæsthesia was observed at first, as in the case of the 0.03 per cent solution, but the rigor of the former experiment was absent in this.

During perfusions with physiological salt solution containing 1 per cent chloralbacid, repeated spasmodic extension of the extremities was the main feature. With the solution containing 0.33 per cent

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TABLE I.

| Number. | Animal. | Solution used. | Total time of perfusion. | Cessation of reflexes. Time after beginning the perfusion. | | | | | Amount of solution perfused. | Red corpuscles at the end of perfusion in fluid from | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Resp. | Skin. | Lid. | Nose. | Heart beat. | | Cannula. | Heart. | Brain. |
| | | | h. m. | h. m. | h. m. | h. m. | h. m. | h. m. | c.c. | | | |
| 1 | Toad | NaCl — 0.6% . . . . . | 6 15 | 4 15 | 5 25 | 5 30 | 6 00 | 6 15 | 475 | + | + | + |
| 2 | " | " " . . . . . | 8 10 | 6 15 | 7 40 | 7 45 | 7 55 | 8 10 | 780 | — | + | + |
| 3 | " | " — 0.8% . . . . . | 3 45 | 2 25 | 3 10 | 3 25 | 3 25 | 3 45 | 290 | + | + | + |
| 4 | " | Ringer's[1] . . . . . . . | 8 30 | 5 25 | 7 25 | 7 45 | 8 05 | 8 30 | 740 | — | + | + |
| 5 | " | " . . . . . . . | 9 10 | 6 00 | 6 15 | 6 15 | 6 30 | 9 10 | 1590 | — | + | + |
| 6 | " | Ringer's[2] . . . . . . . | 4 45 | 2 00 | 3 15 | 3 30 | 3 45 | 4 45 | 625 | + | + | + |
| 7 | " | " . . . . . . . | 3 15 | 2 15 | 2 30 | 2 35 | 2 35 | 3 15 | 575 | + | + | + |
| 8 | Frog | " . . . . . . . | 3 00 | 1 00 | 2 30 | 2 20 | 2 25 | 3 00 | 600 | + | + | + |
| 9 | " | NaCl — 0.6% . . . . . | 3 30 | 2 15 | 2 40 | 2 50 | 2 55 | 3 30 | 275 | . .[3] | . .[3] | . .[3] |
| 10 | " | " " . . . . . | 3 30 | 2 00 | 2 20 | 2 50 | 2 50 | 3 30 | 275 | . .[3] | . .[3] | . .[3] |
| 11 | " | " " . . . . . | 1 20 | 0 30 | 1 00 | 1 15 | 1 15 | 1 20 | 180 | . .[3] | . .[3] | . .[3] |
| 12 | " | {NaCl — 0.7% Calcium saccharate — 0.03%} | 5 50 | 1 10 | 2 45 | 2 50 | 2 55 | . . . .[4] | 650 | — | + | + |
| 13 | " | " " | 8 30 | 2 00 | 3 20 | 3 30 | 3 25 | . . . .[4] | 730 | — | + | + |
| 14 | " | {NaCl — 0.7% Paraxanthin — 0.03%} | 1 25 | 0 35 | 1 00 | 1 10 | 1 05 | 1 25 | 95 | + | + | + |
| 15 | " | {NaCl — 0.7% Paraxanthin — 0.015%} | 2 05 | 0 30 | 1 50 | 1 55 | 1 55 | 2 05 | 145 | + | + | + |
| 16 | " | {NaCl — 0.7% Chloralbacid — 1% . .} | 0 50 | 0 24 | 0 35 | 0 35 | 0 33 | 0 50 | 25 | + | + | + |
| 17 | " | {NaCl — 0.7% Chloralbacid — 0.33%} | 1 05 | 0 29 | 0 36 | 0 50 | 0 52 | 1 05 | 120 | + | + | + |

[1] White's modification: 0.6% NaCl, 0.01% $NaHCO_3$, 0.01% $CaCl_2$, 0.0075% KCl.
[2] Howell's modification: 0.7% NaCl, 0.026% $CaCl_2$, 0.03% KCl.
[3] Not ascertained.
[4] Heart continued to beat long after the conclusion of the experiment.

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of chloralbacid, spasmodic twitching in the limbs was the most noticeable incident.

At the end of the experiments with the solutions containing paraxanthin and chloralbacid, after the heart had ceased to beat, solution of calcium saccharate was perfused. In each case this solution caused the heart to begin beating, and rapidly induced the normal stroke and rhythm.

Before passing to the next series, it should be stated that in each of the preceding experiments the animal became œdematous. Even those animals in which perfusion took place at the lowest possible pressures, and for the shortest intervals, showed unmistakable signs of œdema.

It was impossible to remove entirely the blood-corpuscles from the capillaries in the heart and brain, even when the perfusion was continued uninterruptedly for eight hours, and as much as 1590 c.c. of fluid had slowly passed through the body. In all cases the fluid expressed from the heart and brain contained an appreciable number of red and white corpuscles.

In most of the experiments, when the heart had come to a standstill after continuous irrigation with physiological saline solution, also Ringer's solution, rhythmical contractions could be promptly induced by perfusing Schücking's solution. This result was obtained even when mechanical and electrical stimulation had failed to restore the normal beating.

**Experiments on rabbits.** — We report the results of thirteen experiments on rabbits. In this series we used all of the so-called "indifferent" solutions already mentioned.

Considerable difficulty was experienced in our efforts to devise a method which would prevent almost instant death of the animals, and yet which would speedily result in pronounced anæmia.

Ligaturing the arteries to the brain, before or simultaneously with the beginning of the perfusion, brought on convulsions immediately. This was the case whether the ligatures were placed about the arteries in the neck or in the chest. Even when the perfusion had been begun shortly before the arterial blood was completely shut off, it still remained impossible to prevent convulsions and quickly ensuing death.

In Experiments 1–5 (see the table on page 135), the blood-vessels in the neck were quickly tied as perfusion was begun. In Experiments 6–10, they were tied just above the heart as perfusion was

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instituted. Experiments 11–13 were carried out by the following method.

Instead of closing the arteries to the brain, the abdominal aorta, vena cava, and vena porta were ligated, and the heart's action utilized to pump the perfusion fluid through the brain. The warm solution was directed into the heart by way of one jugular, and passed from the brain by way of the other. With this method, anæmia was gradually though quickly induced, convulsions were entirely prevented, and life was considerably prolonged.

In all cases, microscopic examination of the fluid pressed from the brain showed the presence of red corpuscles.

TABLE II.

| Number. | Weight of Animal. | Solution used. | Total time of perfusion. | Cessation of reflexes. Time after beginning the perfusions. | | | | Amt. of solution perfused. | Pressure of perfused fluid. | Lung œdema. | Blood drawn at beginning of experiment. | Hæmoglobin in exit fluid at end. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Resp. | Lid. | Nose. | Heart beat. | | | | | |
| | gms. | | min. | min. | min. | min. | min. | c.c. | mm. Hg. | | c.c. | per cent. |
| 1 | 1300 | Rabbit serum . . . . . | 17 | 16 | 14 | 14 | 20 | 110 | 90–120 | + | .. | .... |
| 2 | 1100 | NaCl — 1% . . . . . . | 7 | (?) | (?) | (?) | (?) | 30 | 130–150 | + | .. | .... |
| 3 | 1500 | " " . . . . . . | 15 | (?) | 12 | 7 | 18 | 200 | 90 | .. | 15 | .... |
| 4 | 1800 | " " . . . . . . | 9 | 8 | 7 | 6 | 9 | 250 | 90–110 | | 23 | .... |
| 5 | 1600 | " " . . . . . . | 7 | (?) | 2 | 2 | (?) | 35 | 90 | .. | 17 | .... |
| 6 | 1800 | {NaCl — 1%<br>{Calc. sach. — 0.035% | 9 | 4 | 2 | 3 | 6 | 230 | 75–85 | .. | .. | 10–12 |
| 7 | 2800 | " " " | 27 | (?) | 2½ | 4 | 25 | 450 | 110–140 | .. | 9 | 8–11 |
| 8 | 1600 | Rabbit serum . . . . . | 8 | 3 | 3 | 5 | 8 | 110 | 110–150 | .. | .. | .... |
| 9 | 1400 | {NaCl — 1%<br>{Calc. sach. — 0.035% | 2 | (?) | (?) | (?) | (?) | 15 | 100 | + | .. | .... |
| 10 | 1500 | " " " | 8 | (?) | (?) | (?) | (?) | 40 | 100 | + | .. | .... |
| 11 | 1800 | " " " | 20 | 10 | 14 | 16 | 20 | 350 | .... | + | .. | .... |
| 12 | 2000 | NaCl — 1% . . . . . . | 25 | 20 | 13 | 22 | 26 | 260 | 70–110 | .. | .. | 6 |
| 13 | 1900 | Rabbit serum . . . . . | 14 | 11 | 9½ | 12 | 14 | 150 | 80–120 | .. | .. | .... |

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The disappearance of functions in these experiments was not at all regular in the first ten. The events of each experiment transpired so quickly that it was extremely difficult to note accurately the time of cessation of each reflex. In the last three experiments respiration ceased first in one, second in two; the "lid reflex" disappeared first in two, second in one. In each of the last three experiments, the "nose reflex" was the third to disappear. Heart beat was always fourth in the order of cessation.

**Experiments on dogs.** — Only two experiments were performed on dogs. The first was by a method similar to that in the tenth experiment with rabbits. The weight of the dog was 12 kilos. The pressure of perfusion was 140–150 mm. Hg. The amount of blood drawn at the beginning of the experiment was 47 grams. The perfusion fluid was a 0.7 per cent solution of sodium chloride containing 0.03 per cent calcium saccharate. Perfusion was continued for forty-two minutes. The volume of fluid perfused was 1125 c.c. The amount of hæmoglobin present in the fluid leaving the jugular vein at the end of the experiment was 30 per cent of the normal content in blood.

Reflex responses failed in the following order: (1) lid and nose reflexes in twenty-six minutes; (2) respiration in forty minutes; (3) heart beat in forty-two minutes.

There were no convulsions at any stage of the experiment.

In the second experiment, with a small dog weighing only 5 kilos, 200 c.c. of blood was taken, and an equal quantity of horse serum immediately afterwards was transfused to take its place. This process was repeated three times at intervals of half an hour. After the fourth blood-letting, the dog ceased to breathe, and did not recover when the new portion of serum was transfused. Aside from variations in heart action and respiration, no special functional changes were observed until the end, when respiration suddenly ceased, and the other functions came to an end about the same time. Death was neither preceded nor accompanied by convulsions.

## III. Summary of Conclusions.

The more important conclusions of these preliminary experiments are that when the brain is subjected to anæmia by the process of perfusing solutions, such as Ringer's, Schücking's, serum, etc., its functions soon cease. When the anæmia is induced rapidly, convulsions

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ensue. When it is brought about gradually, anæmia may be made acute without causing the appearance of convulsions.

When anæmia of the brain is produced gradually by the methods used in these experiments, the functions here referred to cease usually in the following order:

(A) In cold-blooded animals: (1) respiration, (2) skin reflex, (3) lid reflex, (4) nose reflex, (5) heart beat.

(B) In warm-blooded animals: (1) lid reflex, (2) respiration, (3) nose reflex, (4) heart beat.

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# ON THE FORMATION OF GLYCOGEN FROM GLYCO-PROTEIDS AND OTHER PROTEIDS.

By LYMAN BRUMBAUGH STOOKEY.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

EVER since the original experiments of Claude Bernard on the formation of glycogen from proteids, this theme has been a debated one. The extensive literature on the subject has lately been collected and discussed in a monograph by Cremer,[1] and hence need not be repeated here in detail. Among recent investigators, Schöndorff[2] denies the possibility of direct glycogen formation in the body from proteid substances which fail to yield a typical carbohydrate group on cleavage. In this class are included casein and gelatin, which do not yield reducing bodies when they are decomposed with mineral acids. Schöndorff's negative experiments were carried out on frogs. On the other hand the experiments of Bendix[3] on dogs have led him to the conclusion that in mammals glycogen formation follows the feeding of proteids (like casein) free from a carbohydrate constituent as well as of those (like ovalbumin) which contain the latter in their molecule. In fact, it appears from his protocols that the carbohydrate-free proteids are even better glycogen formers than the glycoproteid ovalbumin; and Bendix suggests that the amido-carbohydrates which are obtainable from the proteids on decomposition, may be unsuited for utilization as carbohydrates in the organism.

Although the formation of sugar from proteid in the organism has long been indicated by the observations of both physiologists and clinicians, and the possibility of glycogen formation from the same source is strongly suggested by the older experiments of Naunyn, v. Mering, Külz, and others, the problem can scarcely be regarded as

[1] CREMER: Ergebnisse der Physiologie, 1902, i, l, p. 803.

[2] SCHÖNDORFF: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 60; 1902, lxxxviii, p. 339.

[3] BENDIX: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 492.



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conclusively solved.[1] The renewed study of the proteids in recent years has made it clear that these substances show far greater differences in chemical structure than was formerly assumed. For many of them it has been possible to demonstrate the existence of a carbohydrate nucleus which is apparently lacking in a smaller number.[2] Accordingly it was to be expected that a reinvestigation of the relation of the purified and better identified proteids to sugar formation in the body might bring new suggestions. Thus the glyconucleoproteid of the pancreas yields a pentose, l-xylose;[3] while chitosamin has been identified as a derivative of other compounds, such as the mucoids, the proteids of cartilage, egg-white, and blood-serum. The 6-carbon carbohydrates which arise from these sources are for the most part nitrogenous; indeed the biological distribution of amido-sugar groups is apparently far more extensive than has heretofore been assumed.

The experiments of Fabian[4] and of Offer and Fraenkel[5] have indicated that chitosamin introduced as such into the body is in great part eliminated again unchanged. Glycogen formation was not obtained. For the glycogenic function of the pentoses the evidence is also still uncertain.[6] But it is conceivable that the behavior of these different carbohydrates when built up in the complex molecule of a proteid may be quite different from that of the isolated cleavage products. The transformation of chitosamin into the non-nitrogenous dextrose is by no means a physiological impossibility. Furthermore Loew[7] has pointed out that, theoretically at least, the formation of glycogen from proteid is conceivable independent of the existence of any preformed carbohydrate group. Synthetic processes may come directly into play.

The present experiments by the writer were undertaken some time ago to determine the influence of true glycoproteids (in the broader

[1] Compare the criticisms of PFLÜGER and his school.

[2] *Cf.* LANGSTEIN: Ergebnisse der Physiologie, 1902, i, l, p. 63.

[3] NEUBERG: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 1467.

[4] FABIAN: Zeitschrift für physiologische Chemie, 1899, xxvii, p. 167.

[5] OFFER and FRAENKEL: Centralblatt für Physiologie, 1899, xiii, p. 489.

[6] *Cf.* CREMER: Zeitschrift für Biologie, 1901, xlii, p. 428; SALKOWSKI: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 393; NEUBERG and WOHLGEMUTH: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 1; FRENTZEL: Archiv für die gesammte Physiologie, 1894, lvi, p. 372.

[7] LOEW: Hofmeister's Beiträge zur chemischen Physiologie, 1902, i, p. 567.

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sense) on glycogen formation. For this purpose the substances under consideration were fed to animals made glycogen-free as far as possible by starvation. A pentose-yielding nucleoproteid of the pancreas, ovomucoid from egg-white, and the so-called "chondrin," or hydrated cartilage, were used. In addition, experiments were conducted with the syntonin of muscle, casein and its salts, and leucin. The trials were all made upon fasting, full-grown hens. In view of the difficulty in obtaining large quantities of thoroughly purified compounds for feeding purposes, the selection of a relatively small animal seemed desirable. The experimental studies of Prausnitz[1] and Hergenhahn[2] have indicated that, after four to six days' starvation, hens are practically free from glycogen — or at any rate that the liver glycogen has usually for the most part disappeared. In the muscles, glycogen may apparently persist for a longer period. How difficult it is to provoke the disappearance of the last traces of glycogen by starvation alone has again been emphasized lately by Pflüger and his co-workers. Indeed it has been suggested that a formation of glycogen may be induced by increased proteid metabolism during starvation. It is difficult, therefore, to make certain of the glycogen content of any animal at the beginning of a feeding trial; and small increases above the average figures for starving animals are to be attributed to the experimental conditions only with great reserve. On the other hand, negative results ought likewise to be interpreted with great caution, wherever the extent of absorption and utilization of the material fed has not been ascertained by appropriate investigations. It is well to point out these limitations of experiments of the character to be described, since they apply to many published researches on this subject.

**Method of experimentation.** — Following an old suggestion of Külz that exposure to cold rapidly decreases the content of liver glycogen in rabbits, the hens were kept without food in a very cold room for at least four days. The substances fed were mixed with a little water and moulded into small pellets of about one gram in weight, in which form they were fed. Water and sand or crushed marble were always available in the feeding periods, during which the hens were again kept at room temperature. When the feeding extended over more

[1] PRAUSNITZ: Zeitschrift für Biologie, 1890, xxvi, p. 377.

[2] HERGENHAHN: Zeitschrift für Biologie, 1890, xxvii, p. 215. KÜLZ found 0.9 per cent in some instances, however. See Centralblatt für Physiologie, 1890, iv, p. 788.

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than one day, a salt mixture containing sodium chloride, sodium phosphate, and ferric chloride was also fed in small quantities. The glycogen was separated by the Brücke-Külz method, and, after hydration to dextrose with 2.2 per cent hydrochloric acid, was estimated by the Allihn gravimetric copper method. In a few cases, estimations were made on muscle tissue removed from the breast; usually the liver alone was examined. The alimentary canal, including the crop, was inspected after the death of the hens, in order to ascertain something regarding the extent to which the materials fed remained unutilized. Control trials indicated that under the conditions stated the glycogen had practically all disappeared from the liver of the starving animals, and only traces were left in the muscles of those studied in this respect. The loss of weight during the starving period was always noted in order to make certain that the animals had not succeeded in obtaining food. They were killed at varying intervals after the feedings, the duration of which was likewise varied. In general fifteen to eighteen hours elapsed after the last feeding.

The preparation and nature of the substances fed may briefly be outlined. *Ovomucoid* was obtained from the concentrated filtrates of coagulated diluted egg-white by precipitation with alcohol. It was redissolved in water, and reprecipitated, until the concentrated washings were sugar-free, as indicated by the absence of any reduction with Fehling's solution. The final sugar-free products readily reduced the latter, after being boiled with mineral acids. Müller and Seeman were able to obtain thirty per cent of glycosamin from ovomucoid.[1] The *glyconucleoproteid of the pancreas* was prepared by comminuting the fresh pancreatic glands of sheep, boiling for fifteen minutes in five times their weight of water, and filtering. From the filtrate, fat was removed after cooling; and acetic acid was added to precipitate the nucleoproteid. The latter was filtered off, washed with water and alcohol, dried, and reduced to a powder. As already mentioned, Neuberg has identified the carbohydrate derivative of the pancreatic nucleoproteid as l-xylose. The "chondrin" fed was prepared by hydration of the carefully cleaned tracheal cartilages of sheep. The material was dried and reduced to a powder. The nature of the reducing substance obtainable from cartilage is somewhat in doubt, although it is generally regarded as identical with chitosamin.[2] *Syntonin* was obtained from thoroughly washed muscle tissue (lean

[1] *Cf.* COHNHEIM: Chemie der Eiweisskörper. 1900, p. 266.
[2] *Cf.* LANGSTEIN: Ergebnisse der Physiologie, 1902, i, l, pp. 80–83.

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| Number of experiment. | Substances fed. | Initial body-weight. | Final body-weight. | Duration of fasting. | Total quantity of substances fed. | Duration of feeding. | Interval between last feeding and death. | Body-weight at death. | Weight of the liver. | Glycogen found in the liver. | | Observations. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | grams. | grams. | da. | grams. | | hrs. | grams. | gms. | grams. | per cent. | |
| I | Ovomucoid | 1469 | 1320 | 4 | 20 | once | 17 | 1345 | 29 | trace | .. | Five gms. ovomucoid recovered from the crop. |
| II | " | 1190 | 1037 | 4 | 20 | once | 20 | 1050 | 18 | trace | .. | Seven gms. ovomucoid recovered from the crop. |
| III | " | 1600 | 1440 | 4 | 18 | once | 24 | 1462 | 24 | trace | .. | Crop and alimentary canal empty. |
| IV | " | 1210 | 960 | 4 | 30 | 2 da. | 14 | 1000 | 20 | 0.05 | 0.25 | Crop and alimentary canal empty. |
| V | " | 1352 | 1160 | 4 | 30 | once | 15 | 1165 | 23 | none | .. | Six gms. ovomucoid recovered from crop. |
| VI | Pancreas nucleoproteid | 1700 | 1535 | 4 | 13 | once | 18 | 1535 | 27 | trace | .. | Crop practically empty; gut normal. |
| VII | " | 1375 | 1140 | 4½ | 60 | 2 da. | 15 | 1170 | 24 | 0.081 | 0.33 | Crop contained only traces of food. |
| VIII | " | 1535 | 1310 | 4 | 30 | once | 15 | 1325 | 27 | none | .. | No food in crop. |
| IX | Chondrin | 1280 | 1100 | 4½ | 30 | once | 18 | 1194 | 22 | 0.134 | 0.61 | No food in crop. |
| X | " | 1305 | 1110 | 4½ | 60 | 2 da. | 18 | 1138 | 25 | 0.04 | 0.16 | No food in crop. |
| XI | " | 1150 | 980 | 4½ | 150 | 5 da. | 18 | 1026 | 20 | 0.048 | 0.24 | Crop contained considerable chondrin; hen appeared unwell on last day. |
| XII | " | 1454 | 1226 | 4 | 30 | once | 15 | 1252 | 23 | none | .. | Crop empty; gut normal. |
| XIII | " | 1150 | 980 | 4½ | 150 | 5 da. | 18 | 992 | 20 | 0.048 | 0.24 | Crop contained considerable chondrin; hen appeared unwell on last day. |
| XVIII | Syntonin | 1400 | 1340 | 5 | 15 | once | 15 | 1350 | 23 | 0.18 | 0.78 | No proteid in the crop. |
| XIX | " | 1600 | 1406 | 5 | 75 | 4 da. | 15 | 1320 | 32 | trace | .. | Crop contained some proteid and the condition of the alimentary tract indicated partial absorption only. The muscle contained 0.7 per cent of glycogen. The hen was ill on the fourth day. |

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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XX | Casein | 1230 | 1097 | 4 | 17 | once | 18 | 1102 | 21 | trace | .. | Only partial absorption. |
| XXI | " | 1366 | 1188 | 5 | 210 | 7 da. | 15 | 1215 | 33 | 1.656 | 5.0 | Traces only of food in alimentary canal. |
| XXII | " | 1610 | 1426 | 5 | 150 | 5 da. | 15 | 1460 | 26 | 0.112 | 0.43 | Incomplete absorption; diarrhœa. |
| XXIII | " | 1370 | 1185 | 5 | 60 | 2 da. | 15 | 1185 | 30 | none | .. | Absorption incomplete; food in crop. |
| XXIV | " | 1275 | 1100 | 4½ | 210 | 7 da. | 15 | 1010 | 27 | none | .. | Imperfect absorption; diarrhœa; food in crop. |
| XXV | " | 1520 | 1250 | 5 | 270 | 9 da. | 15 | 1125 | 30 | none | .. | Partial absorption of food. |
| XXVIII | " | 1160 | 1002 | 4½ | 300 | 10 da. | 15 | 1022 | 21 | 0.65 | 3.1 | Hen vigorous; food evidently utilized. |
| XXIX | " | 1233 | 1077 | 4 | 30 | once | 18 | 1082 | 18 | none | .. | No food residues found. |
| XXX | Sodium casein | 1522 | 1391 | 4 | 30 | once | 16 | 1412 | 28 | none | .. | No food residues observed. |
| XXXI | " | 1100 | 1010 | 4 | 150 | 5 da. | 15 | 1070 | 21 | 0.519 | 2.47 | No food residues observed. |
| XXXII | " | 1223 | 1077 | 4 | 210 | 7 da. | 15 | 1107 | 24 | 0.673 | 2.80 | No food residues observed. |
| XXXIII | " | 1700 | 1570 | 5 | 250 | 9 da. | 15 | 1535 | 23 | 0.641 | 2.78 | The muscle contained 0.9 per cent of glycogen; the crop still contained some food. |
| XXXIV | " | 1270 | 1120 | 5 | 180 | 6 da. | 15 | 1055 | 25 | 0.576 | 2.3 | The muscle contained 0.96 per cent of glycogen; the crop still contained some food. |
| XV | Sodium casein and saccharose | 1400 | 1330 | 5 | 24<br>6 | once | 15 | 1350 | 24 | 0.113 | 0.47 | Food partly unused; hen unwell. |
| XVI | " | 1140 | 1010 | 5 | 24<br>6 | once | 15 | 1025 | 25 | 0.375 | 1.5 | The muscle contained 0.89 per cent of glycogen; no food residues found in alimentary tract. |
| XVII | " | 1035 | 936 | 5 | 24<br>5 | once | 15 | 940 | 24 | 0.394 | 1.64 | The muscle contained 0.7 per cent of glycogen. |
| XXXV | Leucin | 1825 | 1650 | 5 | 28½ | 1½ hr. | 15 | 1680 | 28 | 0.312 | 1.11 | About 10 gms. of leucin were recovered from the crop. The muscle contained 0.77 per cent of glycogen. |
| XXXVI | " | 2060 | 1775 | 5 | 30 | 3 hr. | 18 | 1780 | 30 | 0.493 | 1.64 | Some leucin was recovered from the crop. The muscle contained 0.62 per cent of glycogen. |

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beef) by extraction with dilute hydrochloric acid. It was precipitated from the acid solution by addition of sodium hydroxide, then washed free from alkali and salts, and dried. On account of its insolubility the syntonin was formed into pellets by the addition of a little gelatin to hold together the fine particles. In the preparation of *casein* (caseinogen) a commercial product was treated with hot alcohol and ether to remove fat, and then repeatedly extracted with hot water, until the concentrated washings no longer reduced Fehling's solution. The milk sugar was thus completely removed. To facilitate the formation of pellets for feeding, a few drops of dilute sodium hydroxide solution were added to the powder. The large number of negative and unsuccessful results with this product suggested the preparation of a more soluble salt. *Sodium casein* was accordingly prepared by suspending purified casein in warm water, and adding sodium hydroxide until solution was effected. An alkaline reaction was avoided. The material was evaporated to dryness, pulverized, and fed in the usual way. Casein fails to yield typical carbohydrate derivatives on decomposition. The *leucin* fed was a crude crystalline product obtained by the auto-digestion of pig's pancreas.

**Protocols of experiments.** — The following typical protocol will illustrate the character of the data ascertained in each experiment.

**Experiment IV. Ovomucoid feeding.** — Weight of the hen before starvation: 1210 gm. Duration of starvation: 4 days. Weight after 4 days: 960 gm. Amount of ovomucoid fed over a period of 2 days: 30 gm. The hen was killed 14 hours after the last feeding. Weight: 1000 gm. The crop was practically empty; the condition of the alimentary canal was apparently normal, and suggested that absorption had taken place. Weight of the liver: 20 gm. Glycogen content of the liver: 0.05 gm. = 0.25 per cent.

The more important data have been summarized in the table on pages 142, 143.

**Discussion of the results.** — Earlier experiments[1] have indicated that the glycogen content of the liver of hens reaches its maximum within twelve to twenty-four hours after feeding, and then falls rapidly again, so that at the end of thirty-six hours it may become almost nil. This led in the present trials to the plan of killing the animals within the periods selected above. The marked diminution in body weight noted in every case, and the numerous experiments

[1] *Cf.* Prausnitz: Zeitschrift für Biologie, 1890, xxvi, p. 377.

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which showed only traces of glycogen in the liver, bear evidence of the efficiency of the starvation process in removing this carbohydrate. With reference to the food it may be added that particular care was devoted to obtaining materials which should be free from contaminating sugars or fat. The positive results cannot, therefore, be attributed to the presence of the latter compounds.

The outcome of the feeding experiments with those substances which yield carbohydrate cleavage products — ovomucoid, pancreas nucleoproteid, chondrin — scarcely permits any positive conclusion to be drawn. In some of the more satisfactory trials, where the period of feeding was more prolonged, and the utilization of the food was apparently better, appreciable quantities of glycogen were found. These do not, however, exceed in amount the *maximum* glycogen content (0.97 per cent) which has been observed in the liver of the fasting hen by Külz;[1] although, like the writer, most other investigators have noted very small quantities only. Prausnitz, for example, found 0.06 — 0.13 per cent of glycogen in the liver of the hen after four days' fasting. The feeding of simple proteids — syntonin, casein, and its sodium salts — failed to yield an increase of glycogen in the liver when a single dose was given. But after trials lasting several days, during which considerable quantities of casein were ingested, an accumulation of glycogen too large to be attributed to any residual store in the liver was repeatedly found. (Compare Experiments XXI, XXVIII, XXXI, XXXII, XXXIII, XXIV.) The quantities range from 2.3 to 5 per cent, and were usually obtained in those trials in which the animal remained vigorous and a utilization of the food given was indicated. These observations are thus in accord with the results which Bendix[2] obtained after feeding casein to dogs.

It has already been pointed out that negative results in experiments like the present ones do not necessarily indicate the incapacity of the substances fed to promote glycogen formation. Nothing short of an estimation, in each case, of the actual extent of absorption and metabolism would permit a final interpretation in those cases where glycogen failed to appear in the liver. The experiments (XV, XVI, XVII) in which proteid and sugar were fed simultaneously, were undertaken to see what effect would be produced by quantities of

[1] KÜLZ: Centralblatt für Physiologie, 1890, iv, p. 788. *Cf.* note 2, p. 140.

[2] BENDIX: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 479; 1902, xxxiv, p. 544. Compare the critique of CREMER: Ergebnisse der Physiologie, 1902, i, l, p. 874.

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carbohydrate comparable with those which might be liberated from the glycoproteids in the amounts fed. In two of the three trials the amounts of glycogen found in the liver (1.5, 1.6 per cent) are not inconsiderable, and agree in general with previous experiments with cane sugar.[1] The negative results with the glycoproteids are interesting in view of the similar outcome of the studies by others on chitosamin and pentoses.

With the possibility of sugar- and glycogen-formation in the organism from carbohydrate-free proteids made probable, it is of interest to learn the intermediate stages in this transformation. What constituent groups of the proteid molecule are the antecedents of the newly formed carbohydrate? It has been suggested that the leucins ($C_6H_{13}NO_2$) constitute the most important intermediary products; and it is known that the ordinary proteids yield a large proportion — fifty per cent or more — of leucin on decomposition. The transformation of an amido-acid like leucin into a 6-carbon carbohydrate is not a theoretical impossibility.[2] To test the hypothesis, Cohn[3] fed leucin to starving rabbits. His observations are by no means conclusive, although they point toward an increased glycogen content in the liver; but Simon[4] has repeated the experiments with entirely negative results. It has been pointed out by Bendix[5] that if leucin is an antecedent of glycogen in the body, then all proteids which yield this amido-acid in abundance ought to induce glycogen formation. This is, however, not the case, as the differences between the results of gelatin feeding and casein feeding indicate. The two experiments presented above by the writer do not, at least, speak against the possibility under discussion; nor do they justify any far reaching statements. The physiologist must look forward to a more profound acquaintance with the chemistry of the proteid molecule before the final word can be spoken.

The writer desires to express his obligation to Professor Lafayette B. Mendel for the suggestion of the subject for investigation, and for criticism.

[1] *Cf.* PRAUSNITZ: *Loc. cit.*
[2] *Cf.* MÜLLER: Zeitschrift für Biologie, 1901, xlii, p. 549
[3] COHN: Zeitschrift für physiologische Chemie, 1899, xxviii, p. 211.
[4] SIMON: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 315.
[5] BENDIX: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 492.

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# THE SHARE OF THE CENTRAL VASOMOTOR INNERVATION IN THE VASOCONSTRICTION CAUSED BY INTRAVENOUS INJECTION OF SUPRARENAL EXTRACT.

By S. J. MELTZER and CLARA MELTZER.[1]

[*From the Rockefeller Institute for Medical Research.*]

OLIVER and Schaefer[2] ascribed the vasoconstricting effect of suprarenal extract to its action upon the blood-vessels directly and not to an action upon the vasomotor centres. They based their claim upon the following considerations: first, that a rise of blood-pressure occurs even after section of the cord below the vasomotor centre; second, that a vasoconstriction takes place in a peripheral organ even after that organ is deprived of its nerves; for instance, in the foreleg after the brachial plexus is cut. The validity of the first consideration has already been a matter of discussion. Thus Szymonowicz[3] and Cybulski claimed that in their experiments, section of the cord did interfere with the vasoconstricting effect of the extract. Furthermore, Velich[4] and others called attention to the fact that simple section of the cord does not exclude the influence of all the vasomotor centres of the spinal cord. To meet this requirement, Velich as well as Biedl[5] have thoroughly destroyed or extirpated the entire cord. Both authors report that even under these circumstances suprarenal extract caused a rise of blood-pressure. Velich, however, admits that in many experiments he did not succeed in completely destroying the spinal cord. He had only three successful experiments. In these, however, the effect was apparently not so prompt as in the ones in which the spinal cord was only cut. According to Velich, after destruction of the cord, a much larger dose of the suprarenal extract is required to bring about a similar rise in the blood-pressure, than after simple section of the cord. From this it would

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[2] OLIVER and SCHAEFER: Journal of physiology, 1895, xviii, p. 230.

[3] SZYMONOWICZ: Archiv für die gesammte Physiologie, 1896, lxiv, p. 97.

[4] VELICH: Wiener medizinische Blätter, 1896, xv–xxi, p. 227.

[5] BIEDL: Wiener klinische Wochenscrift, 1896, p. 157.



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appear that the integrity of the cord is indeed a factor in the successful action of the suprarenal extract upon the blood-pressure.

With regard to the second experimental proof of Oliver and Schaefer, namely, that a vasoconstriction takes place in a peripheral organ even after its nerves have been cut, here, too, the objection was raised that not all nerves of these organs had been cut; for instance, section of the brachial plexus does not exclude the entire innervation of the foreleg. But above all, it seems to us that the method which the authors employed is not capable of furnishing conclusive evidence. They enclose a peripheral organ, kidney or forearm, for instance, in a plethysmograph, and assume that each registration of a diminution in the volume of this apparatus means a constriction of the blood-vessels of the organ within it. They themselves state, however, that the constriction of the arterioles can often lead to a dilatation of the larger and middle-sized arteries which will produce an increase in the volume of the plethysmograph. In other words, a constriction of the small arteries may lead either directly to a diminution in the volume of the plethysmograph, or indirectly, by subsequent filling up of the larger vessels, to an increase in the volume. It would seem that under these circumstances we can hardly draw positive conclusions as to the actual condition of the organ from tracings obtained by the plethysmograph. In fact, of the thirteen plethysmographic tracings of the forearm, given by Oliver and Schaefer in their paper, eight show an increase, while only five show a decrease in the volume after intravenous injection of suprarenal extract.

The foregoing analysis shows that the evidence in favor of the theory of Oliver and Schaefer is by no means complete. A further study of this subject by other methods is therefore desirable.

Direct inspection of the blood-vessels has already been mentioned by Oliver and Schaefer as one of the appropriate methods of studying the effect of intravenous injection of suprarenal extract. It occurred to us that the rabbit's ear would be a favorable object for such a study. The condition of the blood-vessels there is easily discernible, and the vasomotor influences of central origin can be satisfactorily removed. Furthermore, the conditions of the blood-vessels of the ear deprived of the vasomotors, can easily be compared at any stage with those of the normal ear; and this can throw light on the share which the central vasomotor mechanism may have in the vasoconstriction caused by the suprarenal extract.

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Such a series of experiments we have made on a large number of animals, and we shall here report our results.

We employed exclusively adrenalin chloride in various dilutions. The injections were made either into the external jugular vein, the femoral vein, the inferior vena cava, or into the marginal vein of one of the ears. In the first series of animals, the cervical sympathetic was cut or resected either on the right or on the left side, and the injections were made either soon after the operation or some time later. The following are a few abbreviated protocols of that series of experiments.

*Experiment XIV. April 23, 1902.* — Brown, medium-sized rabbit.

5.30 P. M. Resected about 2 cm. of cervical sympathetic on left side; vessels of left ear at once very much dilated.

6.28. Injected into marginal vein of right ear 1 c.c. of 1 : 10000 adrenalin.

6.30. Both ears completely blanched.

6.35. Right ear-vessels begin to dilate.

6.42. Same, fully dilated.

6.40. Left ear-vessels begin to dilate, progressing slowly.

7.00. Left returned to normal.

*Experiment XIX. May 7, 1902.* — Large, white rabbit.

4.00 P. M. Left sympathetic resected, both ears very congested. Right femoral vein exposed.

4.23. Injected into vein 1.2 c.c. of 1 : 2000 adrenalin.

4.23.30. Right ear pale.

4.25. Left ear beginning to blanch.

4.28. Fine vessels of right ear begin to appear.

4.29. Vessels of right ear fuller than those of left.

4.36. Left ear still more blanched than before.

4.54. Right ear becomes paler again.

5.45. No change. On taking ether and struggling, the right ear-vessels become very full, left ear remains pale.

*Experiment XXVI. June 19, 1902.* — Brown rabbit, 1500 grammes.

4.50 P. M. Right sympathetic cut.

5.00. Injected into marginal vein of left ear 1.2 c.c. of 1 : 5000 adrenalin.

5.00.30. Left ear pale.

5.01.30. Right beginning to blanch.

5.02. Entire right ear pale, except marginal vein.

5.05.30. Left vessels beginning to dilate.

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*Experiment XXVI — (continued).*

5.08.30. Right central artery becoming very slightly dilated — dilatation not increasing.

5.15. Left ear-vessels, after having been well dilated for about eight minutes, become narrower again.

6.35. No change. Right ear distinctly paler than left.

8.00. No change.

June 20, 9.30 A. M. Vessels of right ear more dilated than those of left, but not as fully dilated as on the previous day soon after operation.

*Experiment XXVII. June 19, 1902.* — Black and white rabbit, 1500 grammes.

5.30. Left sympathetic cut.

5.40. Vessels of left ear very full, of right, medium.

5.41. Injected into right marginal vein 0.5 c.c. of 1 : 5000 adrenalin.

5.41.30. Right entirely pale, except a small central vessel.

5.43. Left beginning to blanch.

5.46. Right getting fuller.

5.47. Left, pallor increasing.

5.55. Left, pale except for large vessels.

6.08. Right vessels as much dilated as before injection.

6.30. Left, still pale.

8.00. Left ear redder than right.

June 20, 9.35 A. M. Left wider than right, but not as fully dilated as after operation.

Many similar experiments were made. The results were the same in every case. The degree of vasoconstriction which was caused by the intravenous injection of adrenalin, was in all cases about the same on the operated as on the normal side. Here we have to mention that Velich reports a single experiment he made on a rabbit in which the right sympathetic nerve was cut, and in which the cadaverous pallor of the ears, following intravenous injection of suprarenal extract, was equal on both sides. In our experiments, however, we have observed some significant differences in the course of the vasoconstriction of the two ears. But before entering upon a detailed discussion of our observations gathered from the above-mentioned series of experiments, we have to report the results of other series of experiments, the execution of which became necessary through the following observation. A study of the effects of cutting and stimulating the third cervical nerve revealed to us[1] its considerable

[1] S. J. and CLARA MELTZER: This journal, 1903, ix, p. 57.

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importance as a vasomotor nerve for the ear, which in many instances exceeds that of the sympathetic nerve. Hence section of the cervical sympathetic nerve alone does not completely deprive the ear-vessels of their central innervation.

The following abbreviated protocols are examples of a series of experiments we made on rabbits, in which the sympathetic as well as the third cervical nerves were cut on the same side.

*Experiment XXIX. Sept. 20, 1902.* — Large gray rabbit.

5.00 P. M. Third cervical and sympathetic cut on the left side. Cannula in inferior vena cava. Animal lost some blood. Left ear-vessels considerably fuller than right.

6.20. Injected through cannula 1 c.c. 1 : 2000 adrenalin. In a few seconds, right ear blanched completely.

6.21. Left ear begins to blanch — blanches slowly.

6.24. Right begins to fill; left still blanching, especially large central vessel.

6.27. Left beginning to fill.

6.30. Both moderately filled, left, a trifle more than right.

6.35. Second injection — 1 c.c. 1 : 2000. In a few seconds right blanched.

6.36. Left begins to blanch — blanches slowly.

6.38. Both begin to fill.

6.45. Third injection, 1.2 c.c. of 1 : 2000. Immediate complete blanching of right ear, followed closely by complete blanching of left ear. Blanching on right side lasted six minutes, then faint filling, which increased to normal within ten minutes; left blanching lasted ten minutes, vessels filled in a few minutes, but not to their former width; blanched and filled again several times. Fourth injection of 1.2 c.c. pure adrenalin (1 : 1000). Complete blanching of right ear in a few seconds, of left, half a minute later. Rabbit killed after fifteen minutes, before ears returned to normal.

*Experiment XXXI. Oct. 5, 1902.* — Brown rabbit.

5.30 P. M. Sympathetic and third cervical cut on the left side.

6.30. Injected through cannula in left jugular vein 1.2 c.c. adrenalin (1 : 1000).

6.30.30. Right ear pale. Fifteen seconds later, left ear beginning to blanch.

6.34. Right beginning to fill; left getting still paler.

6.35. Right, as full as before injection.

6.40. Right, fuller than before injection; left, pale, slight rhythmical dilatation in small area around bifurcation.

6.49. Right, paler again, condition as before injection.

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*Experiment XXXI — (continued).*

7.05. No change in either ear.

8.15. Left ear-vessels dilated again.

8.20. Second injection of 1.2 c.c. undiluted adrenalin. Right ear blanched immediately, half a minute later, left beginning to blanch from below upward.

8.25. Both ears begin to fill, left, slight rhythmical dilatation in the central vessel, while the marginal vessels getting still narrower.

8.28. Right, as before injection.

8.30. Right fuller than before injection.

8.43. Right, again as before injection ; left, still pale.

8.58. Small vessels of left ear becoming visible.

9.45. Central vessel of left somewhat dilated, but not nearly as before injection. Marginal vessel fills up from below.

10.00. Left, almost as full as before injection.

In view of the well-known statement of Claude Bernard[1] that the excision of the superior cervical ganglion causes greater paralytic effects than simple section of the cervical sympathetic nerve, we deemed it desirable to perform a few experiments in which, instead of simple section of the cervical sympathetic, the superior cervical ganglion was removed. The following experiment is an example:

*Experiment XXX. Sept. 22, 1902.* — Large gray rabbit.

5.00. P.M. Ganglion removed, and third cervical cut on the right side. Cannula in inferior vena cava below the renal veins.

6.00. Injected 1 c.c. 1 : 10000 adrenalin.

6.01. Both ears moderately pale.

6.03. Left filling faintly.

6.04.30. Right, central vessel filling from below, rest of ear pale.

6.06. Both ears as before injection.

6.10. Second injection, 0.6 c.c. 1 : 10000 adrenalin.

6.10.30. Left ear getting paler.

6.11.30. Smaller vessels in right beginning to disappear.

6.12. Entire right ear pale.

6.13. Left filling.

6.14. Right filling.

6.15. Right fuller than left.

6.19. Third injection, 1.2 c.c. 1 : 2000 adrenalin.

6.20. Left, entire ear pale ; right, smaller vessels getting narrower.

6.23. Right, central vessel disappearing.

[1] BERNARD : Leçons sur la système nerveux. Paris, 1858, ii, p. 492.

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*Experiment XXX — (continued).*

6.23.30. Left ear getting redder.

6.24. Right, completely pale, left, already a little fuller than before third injection.

6.26.30. Right, central filling slightly.

6.40. Right, not yet as full as before injection.

6.43. Fourth injection, 1.2 c.c. 1 : 2000.

6.44. Left paling.

6.44.30. Right paling.

6.45. Left slightly filling.

6.48. Right beginning to fill.

6.50. Left, as before injection.

6.55. Right, not yet as full as before fourth injection.

In a previous paper[1] we reported that in a few exceptional cases we found that after cutting the branches which connect the third with second and fourth cervical nerves, the corresponding ear became considerably flushed. In a few experiments we have therefore also cut these connections in addition to the removal of the ganglion and cutting of the third cervical nerve. We shall give no protocols of these experiments ; they read the same as the others already quoted. Section of these connecting branches did not change the results in any respect.

We may safely claim that after cutting the third cervical and removing the superior cervical ganglion, the blood-vessels of the ear are deprived of all vasomotor influences of central origin. We know, at least for the present, of no other path by which vasomotors reach the ear. The changes, therefore, which we have seen taking place in the ear-vessels, after the elimination of the above-mentioned nerve and ganglion, can be ascribed only to the influences of some peripheral mechanisms. In all our experiments, without exception, an intravenous injection of a sufficient dose of adrenalin caused a distinct constriction of the blood-vessels of the ear on the side on which the ganglion was removed and the third cervical cut. The constricting effect of adrenalin in these cases is, therefore, certainly of strictly peripheral origin. Furthermore, in all of the experiments in which a dose of adrenalin was employed sufficient to bring out a distinct pallor in the normal ear, there was no case in which the degree of the pallor on the operated side was less than on the normal side. Even when small

[1] S. J. and CLARA MELTZER: This journal, *loc. cit.*, p. 57.

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doses were used, which caused only a moderate constriction of the blood-vessels, lasting a very short time, the degree of the constriction was in both ears the same, provided, of course, the injection was not made into an ear-vein. In the latter case, the ear into which the injection was made has shown the greater pallor.

Our experiments certainly justify the conclusion that suprarenal extract can cause a strong constriction of blood-vessels, which are deprived of all extrinsic neurogenic influences. Is the constriction occurring in normal animals due to the local effect, and to this effect alone? Are the extrinsic vasomotor nerves and centres not affected at all by the suprarenal extract? The fact that the degree of the constriction of the blood-vessels has apparently been the same in both ears, in all cases, would seem to indicate that the central vasomotor mechanism is not a factor in the attainment of the degree of vasoconstriction caused by suprarenal extract. However, our experiments brought to light also some differences in the course of the vasoconstriction in the two ears. These deviations, we believe, are capable of shedding light upon the effect which suprarenal extract might exert upon the central vasomotor mechanisms, and the rôle of the latter in the change of the blood-pressure, which is caused by the extract in the normal part of the animal. The differences relate to the onset, development, duration, and after-effect of the vasoconstriction in the two ears.

1. The most striking point is the difference in the duration of the constriction. In every one of our experiments the constriction of the blood-vessels of the ear on the operated side lasted distinctly longer than that of the ear on the non-operated side. While the duration of the constriction on the normal side rarely exceeded six or seven minutes, and generally amounted only to four or five minutes, we found the constriction on the operated side to last often for hours, and in some instances the vessels did not reach their original size till the following day. In both ears the duration of the constriction depended upon the amount and concentration of the adrenalin we have injected in each case. But even with small quantities and weak solutions, as long as they were capable of producing any degree of constriction, it lasted perceptibly longer on the operated side.

2. Another instructive point is the difference in the mode of the disappearance and the after-effect of the constriction. In the normal ear, after the maximum pallor has lasted a few minutes, the vasoconstriction disappears evenly in a very short time, and is followed by

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a moderate but distinct congestion of the ear. The blood-vessels are then perceptibly wider than before injection. The dilatation lasts a few minutes, its duration being mostly longer than that of the constriction. In the ear on the operated side, we see the maximum constriction, after having lasted for some time, at first giving way only slightly, the larger vessels becoming faintly recognizable. In this state the ear remains stationary again for some time, and then begins to fill up gradually, and very slowly attains its original appearance. In no case have we noticed the ear-vessels of the operated side becoming wider than their original size, not even in such experiments in which the injection was made a long time after the section of the nerves, and the vessels, before the injection of adrenalin, were therefore not much wider on the operated, than on the unoperated side.

3. In all of the experiments, the blanching of the ear on the operated side began distinctly later than on the normal side, provided the injection was not made into the marginal vessel on the operated side, or the dose employed was not too large. The latent period for the normal side is very short, usually only ten to fifteen seconds; it varies slightly with the strength of the dose, and also with the place of injection; for instance, as a rule the constriction appears much sooner after the injection into the jugular vein, than after injection into the inferior vena cava. On the operated side, the latent period was in all instances distinctly longer, the difference varying from fifteen seconds to two minutes.

4. Besides the difference in the length of the latent period, there was invariably also a difference in the time during which the vasoconstriction attained its maximum. In the normal ear there were in all cases not more than a few seconds between the onset of the constriction and the complete blanching of the ear. In the ear on the operated side, the development of the blanching was in nearly all cases a very slow process. It was a frequent occurrence to see the blanching on the operated side still progressing, while on the normal side all signs of a constriction had already completely disappeared.

The length of the latent period and the slow development of the constriction on the operated side cannot be ascribed, at least entirely, to the fact that before the injection the blood-vessels of the ear on that side were more dilated than on the normal side, and therefore more work had to be performed before the same degree of blanching could be attained. The same slow onset and development have been observed in cases in which the injection was made long

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after the operation, and the dilatation was therefore no longer noticeable. They were also present in experiments in which a second injection was made while the ear-vessels on the operated side, as a result of the foregoing injection, were not yet as wide as those on the normal side.

The several differences in the course of the vasoconstriction of the two ears can be ascribed only to the differences in their vasomotor innervation, and indicate clearly that in the normal animal the central vasomotor mechanism plays some part in the causation of constriction of the blood-vessels by intravenous injection of suprarenal extract. As to the interpretation of these differences, we shall discuss first the divergence in the after effect and the duration of the constriction.

The fact that the vessels of the ear on the normal side, after the disappearance of the constriction, become for a while wider than they had been before the injection, can be explained by the assumption that in the normal animal, at a certain phase of the constriction, vasodilating mechanisms are efficiently stimulated. That such a dilatation is not observed on the operated side indicates, furthermore, that the vasodilatation on the normal side is of central origin ; hence, its absence on the operated side. This very assumption also readily explains the difference in the duration of the constriction. The constriction on the normal side is normally abridged, we may assume, by the central stimulation of vasodilators. This stimulation is absent on the operated side, hence the prolonged constriction.

The fact that in many blood-pressure experiments no pressure-lowering after-effect has been observed, is not a serious contradiction to our assumption. The dilatation of the vessels of the normal ear which we have observed as an after-effect was indeed comparatively slight. The main activity of the vasodilatation might consist in reducing to normal the vasoconstriction, which end is perhaps not simultaneously attained in all parts of the body, on account of some differences in the local conditions. In some parts, with an early reduction to normal, there might even occur a slight abnormal dilatation; but in measuring the blood-pressure as a whole, these slight dilatations are liable to be counter-balanced by some still lingering constrictions in other parts. Hence the absence of signs of vasodilatation when studied by the general blood-pressure from the carotid artery, while ocular observation of the vessels of the normal ear

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shows the existence of a moderate vasodilatation as an after-effect in this peripheral organ.

What causes the central stimulation of the vasodilators at a certain phase of vasoconstriction brought on by the suprarenal extract? The hypothesis which appealed to us in the first place is as follows: The suprarenal extract acts on the vasomotors simply as a chemical stimulus. It circulates in the blood in a certain concentration, leaves the capillaries, and attacks the vasomotor centres. There is no reason to believe that the extract has a special affinity for the vasoconstrictors — that it attacks the centre for the vasoconstrictors alone. On the contrary, it is more plausible to assume that it stimulates simultaneously and with equal intensity both antagonistic centres, the centre for vasoconstriction as well as the centre for vasodilatation. It is, however, a well-established fact that when vasoconstrictors and vasodilators are stimulated simultaneously, then a strong stimulus favors the preponderance of vasoconstriction and a weaker stimulus favors the preponderance of vasodilatation. Now a sufficient dose of adrenalin, when first introduced into the circulation, represents of course a strong stimulus, and favors vasoconstriction, hence the immediate constriction of all the vessels of the body and the rise of blood-pressure. After a few minutes, however, the dose of the extract circulating within the blood loses its original strength, either by oxidation of a part of the extract within the blood, or by elimination of a part through the kidneys, or by transudation and deposition of some of the extract into the tissues. The remaining dose now represents a weaker stimulus and favors the preponderance of vasodilatation. We know now from numerous studies that during the preponderance of the activity of one set of nerves, the stimulation of the antagonistic nerves continues in full strength. When, therefore, the dose of adrenalin becomes reduced, and the vasodilators commence to be favored, their activity is immediately in full sway, hence, the rather rapid termination of the constriction and the occasional appearance of a local vasodilatation.

This hypothesis seemed to us to be accessible to an experimental test. If adrenalin stimulate also vasodilators and weak stimuli favor their preponderance, it was reasonable to expect to find a small dose of adrenalin which would cause primarily dilatation of the ear-vessels. We therefore made a large number of experiments in which dilutions varying between 1 : 10000 and 1 : 20000 were injected in quantities of 0.3 to 0.6 c.c. The outcome of these experiments was,

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however, not entirely satisfactory. We succeeded only in a very few instances in obtaining a primary dilatation. The following abridged protocol is an example of such an experiment:

*Experiment XXXII. July 7, 1902.* — Rabbit operated seven days before, right sympathetic resected.

11.58.30 A.M. Injected into marginal vessel of right ear 0.4 c.c. of 1 : 20000 adrenalin. Right ear paled, left ear-vessels moderately dilated and slowly filling up.

12.00. Left ear as full now as just before injection.

12.00.30. Vessels of left ear fully dilated. No initial constriction noted.

12.01. Right ear-vessels slowly filling up again.

The right ear (operated side) became pale in this case through the direct contact of the vessels with the injected adrenalin. The left ear received the extract through the circulation, and the extract was, therefore, very much more diluted. There was no initial constriction, on the contrary, the vessels of the left ear (non-operated side) started to fill up immediately.

In most of the experiments with small doses, however, there was not such an immediate dilatation after the injection. Either there was no change at all, or there was no change for a minute or two, and then a dilatation followed, or finally there was a primary very brief constriction, followed by a pronounced dilatation of the vessels. This series of experiments, though not furnishing an absolute proof, suggests the correctness of the premise in our hypothesis that a smaller dose of the extract favors the preponderance of vasodilatation.

Our hypothesis is based on the assumption that the suprarenal extract has no special affinity for either vasoconstriction or vasodilatation, but represents simply a general nerve stimulus. However, Gürber[1] as well as Hunt[2] stated that they succeeded in obtaining from the suprarenal capsule a substance which causes only vasodilatation. Accordingly, we should have to assume that the extract we employ does not represent one principle which stimulates both centres, but contains separate principles for each of the antagonistic centres. It would offer no difficulty to adapt our hypothesis to this new conception, if it will find general recognition; but we shall abstain from discussing these points for the present.

[1] GÜRBER: Münchener medizinische Wochenschrift, 1897, p. 750.

[2] HUNT: This journal, 1900, iii, p. xviii.

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Regarding the differences in the onset and the development of the vasoconstriction, we wish to emphasize again our contention that these differences are not due entirely to the previous greater engorgement of the ear on the operated side. Our experiments seem rather to demonstrate conclusively that vasoconstriction which is brought about exclusively by peripheral mechanisms, sets in later and develops more slowly than in the case where the peripheral organ has the prompt aid of the central vasomotor mechanisms. As to the reasons for this difference, we can only offer some suggestions. In the first place, it is possible that peripheral mechanisms at all times respond less readily to stimulation than the centres in the cord. There is no lack of analogies for such an assumption. Furthermore, the tonus of the vessels is maintained by continuous excitation by normal stimuli of the vasomotor centres, and not by stimulations of the peripheral mechanisms, as is evident from the fact that cutting of the sympathetic or third cervical nerve causes vasodilatation. The peripheral vasoconstricting mechanisms do not take up the control of the tonus till many weeks after the section of the nerves. It is probable, therefore, that in the normal animal artificial stimuli also affect, in the first place, the central organs, while the peripheral mechanisms are stimulated only secondarily and in lesser degree; hence the tardiness in their response after the elimination of the vasomotor centres. It is possible that the peripheral mechanisms gradually acquire a readiness to respond rapidly to artificial stimulation, just as they gradually acquire the readiness to respond to the continuous normal stimuli for the maintenance of a vascular tonus.

Finally, it is possible that the suprarenal extract in the blood has more obstacles to overcome in reaching the middle coat of the arteries (the muscular layer or the hypothetical ganglia within or around it), than in reaching the vasomotor centres within the cord. To reach the latter the extract has only to pass the endothelial layer of the capillaries which are normally arranged for such passages. While to reach the middle coat of the arteries the extract has to penetrate the complex structure of the arterial intima (endothelial, subepithelial, and elastic layers), which is normally not constructed for purposes of transudation of fluids. As to reaching the middle coat by way of the *vasa vasorum*, we know nothing of the existence of such vessels for the smaller arteries.

However this may be, it is a fact that in blood-vessels which have recently become deprived of their central innervation, the constriction

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sets in late and develops slowly. We have therefore sufficient reason to assume that the rapid onset and development of the constriction in parts with normal central innervation is due to a stimulation by the suprarenal extract of the vasomotor centres; the constriction, however, might be supported in its further course by the peripheral vasoconstricting mechanism which proves to be an efficient though slow agent in the absence of the central innervation.

## Summary.

The removal of the superior cervical ganglion, and section of the the third cervical nerve and its connecting branches on one side of a rabbit, deprives the blood-vessels of the ear on the operated side of all central innervations. The experiments with intravenous injections of adrenalin into rabbits thus operated, brought out the following results :

1. The degree of constriction which the blood-vessels attain in the ear on the operated side is about the same as that of the ear on the normal side.
2. The constriction, however, sets in later and develops more slowly on the operated than on the non-operated side.
3. On the normal side, the constriction is usually followed by a moderate but distinct vasodilatation. Such an after-effect is absent on the operated side.
4. On the operated side, the constriction lasts considerably longer than on the non-operated side.

From these observations, the conclusion is drawn that in the normal animal the injected suprarenal extract stimulates, in the first place, the vasomotor centres. It stimulates the constrictors, as well as the vasodilators; but when the extract is present in the blood in a sufficient dose, it favors constriction, which sets in quite abruptly and develops rapidly. The further continuation of the constriction is possibly also supported by the stimulation of the peripheral mechanism. When, after a few minutes, the dose of the extract within the blood becomes diminished, the stimulation of vasodilatation is now favored, and the constriction therefore soon disappears, giving way in some places even to some degree of vasodilatation. In the absence of central innervation, the vasoconstriction is accomplished by the peripheral mechanisms, which react more slowly to stimulation by the extract, but whose final constricting effect lasts for a considerable time, since it cannot be interrupted by a central vasodilatation.

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# MUSCULAR CONTRACTION AND THE VENOUS BLOOD-FLOW.

By R. BURTON-OPITZ.

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TWO distinct groups of variations in the venous blood-flow are recognizable. The first group embraces those variations in the blood-volume which occur periodically, either with the changes in intra-auricular pressure during each cardiac cycle, or with the changes in intra-thoracic pressure during each respiratory phase.[1] The second group embodies all those variations which are dependent upon accidental, mechanical causes and do not appear at regular intervals. The latter may therefore be termed "irregular" variations.

Leaving out of consideration those obvious changes in the venous flow which result from external mechanical influences, the present paper deals with only the most important class of variations of the latter type; namely, with those produced by the contraction of skeletal muscles.

Briefly outlined, the method consisted in determining quantitatively the volume of the blood-flow, first under normal conditions and subsequently during the different stages of muscular contraction. The femoral vein was used in these experiments, because this vessel is easily isolated and drains a complex of muscles, the nerves of which are readily accessible to the electrodes. The blood-volume was measured by means of Hürthle's recording stromuhr.[2]

The experiments were performed on medium-sized dogs in morphine-ether narcosis. The nerves of the posterior extremity to be experimented on were previously placed in covered electrodes. The sciatic nerve was exposed where it leaves the pelvis, the obturator nerve as it passes across the median surface of the adductor femoralis magnus muscle, and the "crural" nerve at some point of its course along the femoral vessels (saphenous nerve).

[1] BURTON-OPITZ, R: This journal, 1902, vii, pp. 435–459.

[2] HÜRTHLE, C.: Compte rendu du cinquième congrès international de physiologie, Turin, 1901; A short description of this instrument is also given in the paper cited previously.



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The animal was placed upon its back with the posterior extremities slightly flexed and abducted. The toes of the leg experimented on were loosely fastened to a flexible rod.

The femoral vein was isolated from the groin down to the entrance of the vena femoralis posterior superior. In this preparation a very small vein, draining the fatty tissue below the groin, was destroyed. When present, another small vein entering the main vessel nearly opposite the latter was ligated, but those veins found in the immediate neighborhood of the groin were compressed only during the insertion of the stromuhr. The stromuhr, filled with a warm normal saline solution, was placed vertically. Its central cannula came to lie close to the small veins near the groin, while its peripheral cannula remained at some distance from the orifice of the vena femoralis posterior superior.

In those experiments in which the effect of compression of the femoral artery on the blood-flow in the corresponding vein was tried, the artery was placed in a ligature opposite the central cannula of the stromuhr; *i.e.*, about three centimetres below the groin. The artery was raised and compressed between the forceps.

Compression of the femoral vein, peripherally to the orifice of the vena femoralis posterior superior, was also employod. The latter vein drains the largest mass of the gracilis muscle, and thus, by stimulating the obturator nerve, the effect of the contraction of a single muscle on the venous blood-flow could be determined.

Upon the smoked paper of the kymograph were recorded the curve of the blood-flow and the time-curve, written by a Jaquet chronometer in fifths of seconds. The latter record served at the same time as the abscissa of the former. The duration of the stimulation of nerves was marked by an electro-magnetic signal. The respiratory movements were recorded by a tambour communicating with the left pleural cavity. In most of the experiments the venous pressure was also recorded. For this purpose Hürthle's venous manometer was connected by means of a T tube with the peripheral cannula of the stromuhr. The pressure was therefore recorded between the muscles and the instrument.

## The Normal Flow in the Femoral Vein.

It seems advisable to consider first the normal volume of the blood-flow and subsequently the changes which result in consequence of

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muscular contraction. The average value being obtained, a more ready comparison can be made between the normal blood-flow and the flow during a muscular contraction.

The lever of the recording stromuhr of Hürthle writes a continuous curve, composed of upward and downward phases. The writing lever passes in the former direction, if the piston in the central cylinder is driven downward by the blood entering through the opening in the roof of the stromuhr. But if by turning the disc below the floor of the instrument, the blood is forced into the lower part of the cylinder, the piston travels upward and the writing lever records in this case a curve from above downward.

In Tables I to IX the value of the blood-stream is calculated for about one half the total number of phases of each experiment. The duration of each phase and the total quantity of blood propelled during this time having been obtained, these values were reduced to cubic centimetres per second. The latter figures were then employed in calculating the average value of the blood-stream for each experiment.

In order to avoid all errors due to coagulation only about twenty phases were included in the calculation, a number sufficient to obtain a good average. Those variations in the curve, dependent upon the cardiac and respiratory activity,[1] were wholly disregarded; only the duration and height of the entire phase were measured.

As those phases during which compression of the femoral artery was resorted to, would have necessitated a different arrangement of the tables, they are inserted separately after the experiments to which they belong. Those phases, during which stimulation of the nerves of the posterior extremity was tried, are indicated in the tables, but will be considered separately in a later chapter. The last four experiments of this series show the effect of nerve-section on the blood-flow in this particular vein. A sufficient number of normal phases having been recorded, the nerves enumerated above were quickly divided with the scissors, and another series of phases written.

The other details, it seems to me, can easily be derived from Tables I to IX.

[1] Even at such a great distance from the heart the cardiac variations in the blood-flow were often very conspicuous, but naturally their amplitude is less here than in the external jugular vein. The same may be said regarding the respiratory variations.

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TABLE I. EXPERIMENT I.

Weight of dog, 16½ kilos. Left femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total vol. of blood during phase. c.c. | Volume. c.c. per second. | Remarks. |
|---|---|---|---|---|
| 1 | 5.1 | 8.1 | 1.59 | |
| 2 | 5.0 | 7.2 | 1.44 | |
| 3 | 8.6 | 8.7 | 1.01 | |
| 4 | 7.9 | 8.3 | 1.05 | |
| 5 | 7.2 | 7.2 | 1.00 | |
| 6 | 8.0 | 8.0 | 1.00 | |
| 7 | 7.0 | 8.5 | 1.21 | |
| 8 | .. | .. | .. | Compression of femoral artery. |
| 9 | 8.3 | 6.9 | 0.83 | |
| 10 | .. | .. | .. | Compression of femoral artery. |
| 11 | 7.9 | 9.0 | 1.14 | |
| 12 | 8.0 | 8.0 | 1.00 | |
| 13 | 8.2 | 8.1 | 0.98 | |
| 14 | 5.4 | 5.4 | 1.00 | |
| 15 | .. | .. | .. | Compression of femoral artery. |
| 16 | 9.1 | 8.3 | 0.91 | |
| 17–19 | .. | .. | .. | Stimulation of sciatic nerve, tetanic current. |
| 20 | .. | .. | .. | Compression of femoral artery. |
| 21 | 7.2 | 8.0 | 1.11 | |
| 22 | 7.8 | 8.3 | 1.06 | |
| 23 | 8.2 | 7.8 | 0.95 | |

Highest value, 1.59; lowest value, 0.83; average value, 1.08 c.c. per sec.

EXPERIMENT I. Compression of the femoral artery.

| No. of phase. | Duration of compression in seconds. | Total quantity of blood during compression. c.c. | Volume. c.c. per second. | Average value. c.c. per second. | Av. value of normal blood-flow. c.c. per second. | Decreases during compression. Per cent. |
|---|---|---|---|---|---|---|
| 8 | 5.2 | 0.47 | 0.09 | 0.10 | 1.08 | 90 |
| 10 | 12.1 | 1.28 | 0.10 | | | |
| 15 | 11.6 | 1.18 | 0.10 | | | |
| 20 | 6.7 | 0.89 | 0.13 | | | |

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TABLE II. EXPERIMENT II.

Weight of dog, 13 kilos. Left femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total vol. of blood during phase. c.c. | Volume. c.c. per second. | Remarks. |
|---|---|---|---|---|
| 1 | 7.2 | 6.0 | 0.83 | |
| 2 | 14.5 | 10.8 | 0.75 | |
| 3 | 13.1 | 11.5 | 0.87 | |
| 4 | .. | .. | .. | Compression of femoral artery. |
| 5 | 12.8 | 10.1 | 0.79 | |
| 6 | 14.9 | 10.8 | 0.72 | |
| 7 | 13.6 | 9.0 | 0.66 | |
| 8 | .. | .. | .. | Compression of femoral artery. |
| 9 | 12.5 | 9.0 | 0.72 | |
| 10a | .. | .. | .. | Compression of femoral vein. |
| 10b | .. | .. | .. | Stimulation of obturator nerve, tetanic current. |
| 11a | .. | .. | .. | Compression of femoral vein. |
| 11b | .. | .. | .. | Stimulation of obturator nerve, tetanic current. |
| 12 | 12.3 | 10.6 | 0.86 | |
| 13 | 14.5 | 11.3 | 0.77 | |
| 14 | 13.6 | 12.1 | 0.88 | |
| 15 | 13.9 | 12.0 | 0.86 | |
| 16 | 15.9 | 11.0 | 0.69 | |
| 17–20 | .. | .. | .. | Stimulation of sciatic nerve, tetanic current. |
| 21 | 13.6 | 9.7 | 0.71 | |
| 22 | 12.3 | 9.5 | 0.77 | |

Highest value, 0.88; lowest value, 0.66; average value, 0.77 c.c. per sec.

EXPERIMENT II. Compression of femoral artery.

| No. of phase. | Duration of compression in seconds. | Total vol. of blood during compression. c.c. | Volume. c.c. per second. | Average value. c.c. per second. | Av. value of normal blood-flow. c.c. per second. | Decrease during compression. Per cent. |
|---|---|---|---|---|---|---|
| 4 | 8.1 | 1.3 | 0.16 | 0.18 | 0.77 | 76 |
| 8 | 10.7 | 2.2 | 0.20 | | | |

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TABLE III. EXPERIMENT III.

Weight of dog, 14 kilos. Right femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total vol. of blood during phase. c.c. | Volume. c.c. per second. | Remarks. |
|---|---|---|---|---|
| 1 | 19.6 | 11.8 | 0.60 | |
| 2 | 20.5 | 11.6 | 0.56 | |
| 3 | .. | .. | .. | Compression of femoral artery. |
| 4 | 17.5 | 11.1 | 0.63 | |
| 5 | 17.0 | 12.1 | 0.71 | |
| 6 | 16.0 | 9.2 | 0.57 | |
| 7a | .. | .. | .. | Compression of femoral vein. |
| 7b | .. | .. | .. | Stimulation of obturator nerve, single induction. |
| 8 | 15.0 | 8.7 | 0.58 | |
| 9 | 14.3 | 10.5 | 0.73 | |
| 10 | 18.7 | 9.2 | 0.49 | |
| 11 | .. | .. | .. | Stimulation of sciatic nerve, single induction. |
| 12 | 16.1 | 11.0 | 0.68 | |
| 13 | 16.9 | 11.9 | 0.70 | |
| 14 | .. | .. | .. | Stimulation of sciatic nerve, single induction. |
| 15 | 14.0 | 10.9 | 0.77 | |
| 16 | 15.3 | 9.7 | 0.63 | |
| 17a | .. | .. | .. | Compression of femoral vein. |
| 17b | .. | .. | .. | Stimulation of obturator nerve, tetanic current. |
| 18 | 18.2 | 10.6 | 0.58 | |
| 19 | 17.2 | 10.0 | 0.57 | |
| 20 | .. | .. | .. | Compression of femoral artery. |
| 21 | .. | .. | .. | Stimulation of sciatic nerve, tetanic current. |
| 22 | .. | .. | .. | Compression of femoral artery. |

Highest value, 0.77; lowest value, 0.49; average value, 0.63 c.c. per sec.

EXPERIMENT III. Compression of femoral artery.

| No. of phase. | Duration of compression in seconds. | Total vol. of blood during compression. c.c. | Volume. c.c. per second. | Average value. c c. per second. | Av. value of normal blood-flow. c.c. per sec. | Decrease dur. compression. Per cent. |
|---|---|---|---|---|---|---|
| 3 | 5.2 | 1.3 | 0.25 | | | |
| 20 | 5.4 | 1.4 | 0.26 | 0.23 | 0.63 | 63 |
| 22 | 5.3 | 1.1 | 0.20 | | | |

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TABLE IV. EXPERIMENT IV.

Weight of dog, 12½ kilos. Left femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total vol. of blood during phase. c.c. | Volume. c.c. per second. | Remarks. |
|---|---|---|---|---|
| 1 | 14.0 | 9.1 | 0.65 | |
| 2 | 11.8 | 9.6 | 0.81 | |
| 3 | .. | .. | .. | Compression of femoral artery. |
| 4 | 11.5 | 9.6 | 0.83 | |
| 5 | 11.6 | 8.2 | 0.70 | |
| 6a | .. | .. | .. | Compression of femoral vein. |
| 6b | .. | .. | .. | Stimulation of obturator nerve, single induction. |
| 7 | 13 5 | 9 1 | 0.67 | |
| 8 | 12.4 | 8.6 | 0.69 | |
| 9 | 10 5 | 9.2 | 0.87 | |
| 10 | .. | .. | .. | Stimulation of sciatic nerve, single induction. |
| 11 | 11.5 | 8.4 | 0.73 | |
| 12 | .. | ... | .. | Stimulation of sciatic nerve, single induction. |
| 13 | 13.2 | 8.5 | 0.64 | |
| 14 | 11.7 | 8.2 | 0 70 | |
| 15 | 13.0 | 9.0 | 0 69 | |
| 16 | .. | .. | .. | Stimulation of sciatic nerve, tetanic current. |
| 17 | 15.0 | 11.0 | 0 73 | |
| 18 | .. | .. | .. | Stimulation of sciatic nerve, tetanic current. |
| 19 | 12.8 | 11.0 | 0.86 | |
| 20 | 14.4 | 10.1 | 0.70 | |
| 21 | 12.9 | 9 7 | 0.75 | |
| 22a | .. | .. | .. | Compression of femoral vein. |
| 22b | .. | .. | .. | Stimulation of obturator nerve, tetanic current. |
| 23 | 7.0 | 5.4 | 0.77 | |

Highest value, 0.87; lowest value, 0.64; average value, 0.73 c.c. per sec.

EXPERIMENT IV. Compression of femoral artery.

| No. of phase. | Duration of compression in seconds. | Total vol. of blood during compression. c.c. | Volume. c.c. per second. | Average value. c.c. per sec. | Av. value of normal blood-flow. c.c. per sec. | Decrease dur. compression. Per cent. |
|---|---|---|---|---|---|---|
| 3 | 14.7 | 3.2 | 0.21 | 0.21 | 0.73 | 71 |

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TABLE V. EXPERIMENT V.

Weight of dog, 17 kilos. Right femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total volume of blood during phase. c.c. | Volume. c.c. per sec. | Remarks. |
|---|---|---|---|---|
| 1 | 6.5 | 7.7 | 1.18 | |
| 2 | 6.4 | 8.3 | 1.29 | |
| 3 | 7.5 | 8.2 | 1.09 | |
| 4 | 7.9 | 9.5 | 1.20 | |
| 5 | 8.1 | 9.0 | 1.11 | |
| 6 | .. | .. | .. | Stimulation of sciatic nerve, single induction. |
| 7 | 6.9 | 8.7 | 1.26 | |
| 8 | 5.8 | 8.0 | 1.38 | |
| 9 | 6.2 | 8.0 | 1.29 | |
| 10 | 6.9 | 9.0 | 1.30 | |
| 11 | 7.8 | 9.1 | 1.16 | |
| 12 | 6.7 | 8.0 | 1.19 | |
| 13 | 8.1 | 8.7 | 1 07 | |
| 14 | 8.8 | 9.1 | 1.03 | |
| 15 | .. | .. | .. | Stimulation of sciatic nerve, single induction. |
| 16 | 6.2 | 7.6 | 1.22 | |
| 17 | .. | .. | .. | Stimulation of sciatic nerve, single induction. |
| 18 | 7.0 | 7.9 | 1.12 | |
| 19 | 7.2 | 8.5 | 1.18 | |
| 20 | 8.0 | 8 6 | 1.07 | |
| 21 | 8.0 | 9.0 | 1 12 | |

Highest value, 1.38; lowest value, 1.03; average value, 1.18 c.c. per sec.

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TABLE VI. EXPERIMENT VI.

Weight of dog, 14½ kilos. Left femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total volume of blood during phase. c.c. | Volume. c.c. per sec. | Remarks. |
|---|---|---|---|---|
| 1 | 7.1 | 5.6 | 0.79 | |
| 2 | 10.1 | 8.5 | 0.84 | |
| 3 | 5.5 | 6.9 | 1.25 | |
| 4 | 9.7 | 12.1 | 1.24 | |
| 5 | 10.1 | 10.2 | 1.00 | |
| 6 | 8.1 | 9.1 | 1.12 | |
| 7 | 9.5 | 10.4 | 1.09 | |
| 8 | 8.2 | 7.7 | 0.93 | |
| 9 | 10.5 | 11.4 | 1.08 | |
| 10–13 | .. | .. | .. | Stimulation of sciatic nerve, tetanic current. |
| 14 | 9.8 | 12.7 | 1.29 | |
| 15 | 9.1 | 10.0 | 1.09 | |
| Normal flow: highest value, 1.29; lowest value, 0.79; *average value*, 1.06 c.c. per sec. | | | | |
| 16–17 | .. | .. | .. | *Sciatic, obturator, and crural nerves cut.* |
| 18 | 3.2 | 11.8 | 3.6 | |
| 19 | 4.2 | 12.1 | 2.8 | |
| 20 | 4.1 | 10.2 | 2.4 | |
| 21 | 3.2 | 12.2 | 3.8 | |
| 22 | 3.4 | 9.5 | 2.8 | |
| 23 | 3.0 | 12.0 | 4.0 | |
| 24 | 3 6 | 11.6 | 3.2 | |
| 25 | 4.1 | 11.4 | 2.7 | |
| 26 | 3.8 | 11.6 | 3.0 | |
| Flow after nerve-section: highest value, 4.0; lowest value, 2.4; *average value*, 3.1 c.c. per sec. | | | | |

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TABLE VII. EXPERIMENT VII.

Weight of dog, 12 kilos. Left femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total volume of blood during phase. c.c. | Volume. c.c. per sec. | Remarks. |
|---|---|---|---|---|
| 1 | 19.3 | 10.0 | 0.52 | |
| 2 | 25.0 | 12.2 | 0.49 | |
| 3 | .. | .. | .. | Stimulation of sciatic and obturator nerves, tetanic. |
| 4 | 19.3 | 11.2 | 0.58 | |
| 5 | 18.0 | 12.4 | 0.68 | |
| 6 | 20.5 | 11.6 | 0.56 | |
| 7 | 22.2 | 10.3 | 0.46 | |
| 8 | .. | .. | .. | Stimulation of obturator nerve, tetanic. |
| 9 | 21.7 | 12.0 | 0.55 | |
| 10 | 20.1 | 12.5 | 0.62 | |
| 11 | .. | .. | .. | Stimulation of sciatic and obturator nerves, tetanic. |
| 12 | 20.9 | 10.8 | 0.51 | |
| Normal flow: highest value, 0.68; lowest value, 0.46; *average value*, 0.55 c.c. per sec. | | | | |
| 13–14 | .. | .. | .. | *Sciatic and obturator nerves cut.* |
| 15 | 8.3 | 12.6 | 1.51 | |
| 16 | 7.3 | 10.2 | 1.39 | |
| 17 | 7.9 | 10.7 | 1.35 | |
| 18 | 6.4 | 11.3 | 1.76 | |
| 19 | 8.0 | 10.1 | 1.26 | |
| 20 | 5.3 | 7.7 | 1.45 | |
| 21 | 7.5 | 11.7 | 1.55 | |
| 22 | 6.4 | 9.0 | 1.40 | |
| 23 | 6.4 | 9.1 | 1.42 | |
| 24 | 4.5 | 7.8 | 1.51 | |

Flow after nerve-section: highest value, 1.76; lowest value, 1.26; *average value*, 1.46 c.c. per sec.

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TABLE VIII. EXPERIMENT VIII.

Weight of dog, 18 kilos. Left femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total volume of blood during phase. c.c. | Volume. c.c. per sec. | Remarks. |
|---|---|---|---|---|
| 1 | 8.7 | 10.6 | 1.21 | |
| 2 | 9.2 | 10.2 | 1.10 | |
| 3 | 9.5 | 11.5 | 1.21 | |
| 4 | 7.1 | 11.1 | 1.56 | |
| 5 | 9.5 | 12.0 | 1.26 | |
| 6 | 10.2 | 10.5 | 1.02 | |
| 7 | 8.1 | 9.0 | 1.11 | |
| 8 | 9.3 | 10.8 | 1.16 | |
| 9–13 | .. | .. | .. | Stimulation of nerves, single induction. |
| 14 | 9.4 | 9.5 | 1.01 | |
| 15 | 8.3 | 11.4 | 1.37 | |
| Normal flow: highest value, 1.56; lowest value, 1.01; *average value,* 1.20 c.c. per sec. | | | | |
| 16–18 | .. | .. | .. | *Sciatic, obturator, and crural nerves cut.* |
| 19 | 3.7 | 11.9 | 3.21 | |
| 20 | 2.7 | 10.1 | 3.73 | |
| 21 | 3.4 | 10.2 | 3.00 | |
| 22 | 2.8 | 8.2 | 2.92 | |
| 23 | 3.8 | 11.7 | 3.07 | |
| 24 | 3.2 | 10.0 | 3.12 | |
| 25 | 3.5 | 12.1 | 3.45 | |
| 26 | 3.0 | 10.5 | 3.50 | |
| 27 | 3.2 | 9.8 | 3.06 | |
| 28 | 3.6 | 10.6 | 2.94 | |
| Flow after nerve-section: highest value, 3.73; lowest value, 2.92; *average value,* 3.20 c.c. per sec. | | | | |

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TABLE IX. EXPERIMENT IX.

Weight of dog, 11 kilos. Right femoral vein used.

| No. of phase. | Duration of phase in seconds. | Total volume of blood during phase. c.c. | Volume. c.c. per sec. | Remarks. |
|---|---|---|---|---|
| 1 | 18.6 | 9.5 | 0.51 | |
| 2 | 21.7 | 10.0 | 0.46 | |
| 3 | 20.4 | 10.2 | 0.50 | |
| 4 | 19.1 | 11.5 | 0.60 | |
| 5 | .. | .. | .. | Stim. of sciatic and obturator nerves, tetanic current. |
| 6 | 22.0 | 10.8 | 0.49 | |
| 7 | 25.4 | 12.0 | 0.47 | |
| Normal flow: highest value, 0.60; lowest value, 0.46; *average value*, 0.50 c.c. per sec. | | | | |
| 8–9 | .. | .. | .. | *Sciatic and obturator nerves cut.* |
| 10 | 6.4 | 8.7 | 1.35 | |
| 11 | 5.9 | 10.3 | 1.74 | |
| 12 | 6.2 | 10.7 | 1.72 | |
| 13 | 5.9 | 9.5 | 1.61 | |
| 14 | 6.3 | 10.5 | 1.66 | |
| 15 | 7.2 | 11.1 | 1.54 | |
| 16 | 7.3 | 10.8 | 1.47 | |
| Flow after nerve section: highest value, 1.74; lowest value, 1.35; *average value*, 1.58 c.c. per sec. | | | | |

The most important facts derived from Tables I to IX are more comprehensively arranged in Table X. This outline, however, also includes the velocity of the blood-stream for five of the experiments, this value being given in millimetres per second.

In obtaining the internal diameter of the vein, necessary in this calculation, I have again employed the method which Tschuewsky[1]

[1] Tschuewsky, F. A.: О Кровоснабженіи Отдѣльныхъ Органовъ (On the blood-supply of several organs), Charkow, 1902.

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made use of in his investigation on the blood-flow in different arteries. Although far from being exact, it was the most suitable for these experiments. The outside diameter of the vein having been determined by means of calipers, the vein was lightly compressed between two narrow plates of glass. The thickness of the glass-plates being deducted from this measurement gives the thickness of the vessel wall, which in turn is deducted from the outside diameter of the vein.

TABLE X.

THE FLOW OF THE BLOOD IN THE FEMORAL VEIN.

| Experiment. | Weight of dog. Kilos. | A. Normal volume of blood. c.c. per sec. | B. Volume during compression of femoral artery. c.c. per sec. | Per cent of decrease during compression. | C. Volume after section of nerves. c.c. per sec. | Increase C. times A. | Internal diameter of vein in mm. | D. Velocity of blood stream. mm. per sec. |
|---|---|---|---|---|---|---|---|---|
| 1 | 16.5 | 1.08 | 0.10 | 90 | .. | .. | 4.3 | 74.7 |
| 2 | 13 | 0.77 | 0.18 | 76 | .. | .. | 4.0 | 61.3 |
| 3 | 14 | 0.63 | 0.23 | 63 | .. | .. | .. | .. |
| 4 | 12.5 | 0.73 | 0.21 | 71 | .. | .. | .. | .. |
| 5 | 17 | 1.18 | .. | .. | .. | .. | 5.0 | 60.1 |
| 6 | 14.5 | 1.06 | .. | .. | 3.10 | 2.9 | .. | .. |
| 7 | 12 | 0.55 | .. | .. | 1.46 | 2.6 | 3.8 | 48.5 |
| 8 | 18 | 1.20 | .. | .. | 3.20 | 2.6 | 4.9 | 63.7 |
| 9 | 11 | 0.50 | .. | .. | 1.58 | 3.1 | .. | .. |
| Av. values. | 14.2 | 0.85 | 0.18 | 75 | 2.33 | 2.8 | 4.4 | 61.6 |

Among the conclusions derived from the preceding table the following may be emphasized. We observe first that in spite of the large calibre of the vein, the blood-volume is rather small. It varies from 0.50 c.c. per second in a dog, weighing 11 kilos, to 1.20 c.c. per second in a dog, weighing 18 kilos. The average value of the blood-flow, as obtained in the above nine experiments, is 0.85 c.c. per second; the average weight, 14.2 kilos.

Although the correspondence between the weight of the animal and the blood-volume is not brought out very strikingly in these ex-

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periments, the table at least strongly suggests that in general the volume of the blood-flow increases and decreases in proportion to the weight of the animal.

If a comparison is made between the volume of the blood-stream in the femoral and that in the external jugular vein, it is found that the former is considerably smaller. The eight experiments[1] which I made to determine the normal quantity of blood in the right external jugular vein, gave the average value of 2.03 c.c. per second. The femoral vein, therefore, carries less than one half this amount of blood.

It cannot be assumed that this difference in the blood-volume of the veins under consideration is due to corresponding differences in body-weight, because the average weight of the animals used in determining the blood-flow in the external jugular vein was 12 kilos, while that of the dogs used in the present experiments is 14.2 kilos. Although smaller than the external jugular, the lumen of the femoral vein is of considerable size, at least its large internal diameter is not proportionate to the small quantity of blood traversing this vessel.

In the pamphlet referred to previously Tschuewsky gives a series of experiments on the blood-flow in the femoral artery. The average value of the blood-volume in seven dogs, ranging in weight from 12.5 to 14.5 kilos, is 0.63 c.c. per second. Two other dogs, weighing 37.0 and 51.0 kilos respectively, showed a flow of 1.2 c.c. and 1.0 c.c. per second. If the average value for the venous flow (0.85 c.c. per second) is compared with that of the arterial (0.65 c.c. per second), it is found to be slightly greater. The difference, amounting to only 0.2 c.c. per second, could easily be explained by referring to the differences in the weight of the dogs; those used in the present experiments being the heavier. However, it is also noticed that in the vein the maximal value of 1.0–1.2 c.c. per second was obtained already in dogs weighing only 16 to 18 kilos. These facts suggest that even normally the volume of blood traversing the vein is slightly greater than that of the corresponding artery. It is, however, not absolutely correct to draw these conclusions from two different sets of experiments, or animals.

In support of the previous statement the following fact might be cited: If the femoral artery is compressed opposite the stromuhr, the blood-flow in the vein does not cease completely. The quantity of

[1] BURTON-OPITZ: This journal, 1902, vii, Experiments 1 to 5, on page 439; Experiment 2, on page 441; and Experiments 1 and 2, on page 442.

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blood still propelled must therefore reach this vein in an indirect way by means of anastomosing vessels. It is possible that even normally a slight quantity of blood reaches the vein in this manner.

The reduction in the volume of the blood-stream, produced by the procedure just mentioned, is not uniform. In the experiments given above the decrease varied from 63 to 90 per cent. In another experiment not inserted above, because the record was accidentally destroyed, the decrease amounted to only 51 per cent. Thus, the compression caused in this case a reduction in the blood-flow of only about one half its former volume.

From what has been said regarding the normal blood-flow and the diameter of the femoral vein, it can readily be concluded that the velocity of the current is very slight. The five experiments inserted above have given an average value of 61.6 mm. per second. If the velocity of the venous current is compared with that in the corresponding artery (134.4 mm. per second)[1] it is found to be about one half as great.

After section of the nerves innervating the posterior extremity, the phases written by the lever of the stromuhr immediately became much steeper, indicating thereby that a greater quantity of blood traversed the vein. In the Experiments VI to IX in which this procedure was tried the resulting flow was from 2.6 to 3.1 times greater than the normal (average 2.8 times greater).

A comparison between the quantitative determinations of the venous and arterial blood-flow shows that the insertion of the stromuhr into a vein is not such a serious procedure as might be supposed at first. At least, if any impediment to the venous circulation is produced thereby, it is not greater than that which results if this instrument is placed in an artery.

## The Changes in the Normal Flow produced by Muscular Contractions.

**Tetanic current.** — In the preceding paragraphs we have determined the volume of the blood-flow when the muscles of the posterior extremity are at rest. The present chapter contains a consideration of those changes in the blood-flow which ensue when muscular contractions occur.

[1] See Tschuewsky's paper, page 76.

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The sciatic and obturator nerves were stimulated while the curve of the blood-flow was being written. The stimulation was confined to one, or included both the nerves enumerated. Both tetanic and single induced currents were used, their strength being varied so as to produce either a strong, or a medium muscular contraction. The phases during which stimulation of the nerves was resorted to are indicated in the tables inserted previously.

Whether only one or both nerves were stimulated, the general characteristics of the variation in the blood-flow remained always the same for each kind of stimulus. Changes in the intensity of the cur-

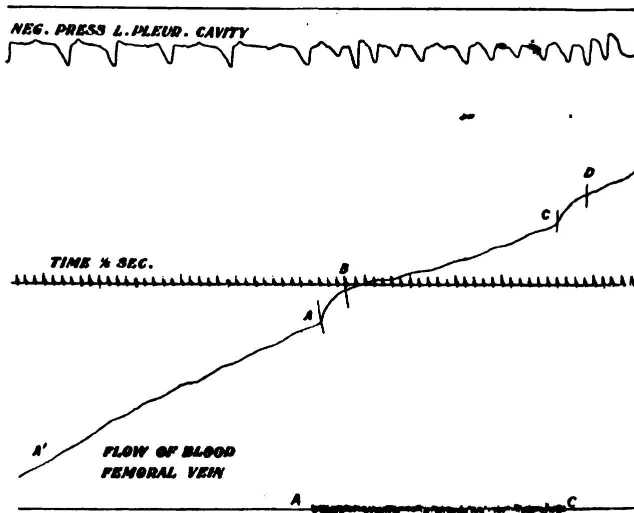


FIGURE 1. — Two-thirds the original size. Variation in the venous blood-flow during a tetanic muscular contraction.

rent also produced no alterations in the general outline of the curve.. The only difference noticed under all conditions was merely one of one degree; *i. e.*, the more nerves stimulated and the stronger the current, the more evident was the variation in the blood-flow.

It seems advisable to consider first the details of a variation produced by a tetanic muscular contraction. For this purpose Fig. 1 is inserted in this place. A tetanizing current of medium strength (distance of coils, 13 cm.) was in this case applied to the sciatic nerve; duration of stimulation, about six seconds.

We observe immediately that the curve of the blood-flow (from *a′*

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to *a*) becomes very steep at *a*, suggesting thereby that a great increase in the volume of the blood-stream has taken place. When the ordinates are compared, this point is found to correspond with the moment of stimulation. At *b* the opposite effect is noticeable. The decrease in the flow beginning here continues to *c*, to the moment of breaking the current. Furthermore, it is evident that the flow becomes greater than normal immediately on discontinuing the stimulation; the curve showing for a brief period a greater incline than the normal (*c* to *d*). Eventually, however, the blood-flow returns to its normal value.

The period of great onward movement, occurring after the application of the current (*a* to *b*), is therefore synchronous with the period of muscular shortening. It is noticed, moreover, that the quantity of blood forced into the vein is greater during the first part of this period than during its latter half, when the muscles have nearly reached their maximal degree of contraction. Apparently, this increase in the blood-volume is due solely to the pressure of the contracting muscular substance upon the mass of blood contained in its vessels.

As soon as the point of maximal shortening has been reached (*b*) the curve inclines strongly toward the abscissa, indicating thereby that the blood-flow is less than normal (*b* to *c*). The decrease in the flow is most conspicuous during the first part of this period, while, if the stimulation is continued for a longer time, a slight and gradual increase above the previous value is noticeable during its latter half. This slight rise becomes the more evident, the longer the muscles are kept in the contracted state. The tetanic muscle therefore is an obstacle to the blood-flow, but when in the course of a longer stimulation the muscle becomes relaxed by fatigue, a steadily increasing quantity of blood is enabled to pass.

The complete relaxation of the muscles on breaking the current is followed by a brief period of increased venous flow (*c* to *d*), after which the normal value is again slowly established. Considered quantitatively, this gush-like rise, after removing the hindrance to the blood-flow, is always much smaller than that occurring during the shortening of the muscles (*a* to *b*).

The entire variation in the venous blood-flow produced by a tetanic muscular contraction may therefore be divided into the following phases:

1. Period of great flow, synchronous with the muscular shortening (*a* to *b*).

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2. Period of slight flow, continuing during the contracted state of the muscle (*b* to *c*).

3. Short period of increased flow, following the relaxation of the muscle (*c* to *d*).

The question how the venous blood-flow is altered by a tetanic muscular contraction has previously been investigated by Sadler[1] and subsequently by Gaskell.[2] The latter author enlarged upon the work of the former by bringing the changes in the blood-volume into relation with the alterations in the form of the muscle. He isolated the vein, draining the largest mass of the musculus vasti and musculus cruralis and measured by a special device the quantity of blood escaping from this vessel, before and during the contraction of the muscles mentioned. As far as the general characteristics of the changes observed by Gaskell are concerned, the results obtained by means of the stromuhr completely substantiate those found by the method just cited.

To show that the retardation of the blood-stream following the maximal muscular shortening (*b* to *c*) is not due to the compression of the larger vessels between the entire complex of muscles, but is caused by mechanical obstacles within each muscle, the following experiment was repeatedly tried. Both the arterial and venous trunks were isolated from all surrounding tissues from the groin downward to the musculus gracilis. The femoral vein was then compressed peripherally to the orifice of the vena musculus gracilis, so that the stromuhr recorded only the flow through this vessel. The musculus gracilis was tetanized by stimulation of the obturator nerve.

The variations in the blood-flow accompanying the tetanization of the gracilis muscle showed under these conditions the same outline as those obtained when the total quantity of blood was measured during the contraction of the entire posterior extremity. However, as the volume of blood was in this case much smaller, it naturally follows that the amplitude of the variations was much less.

In localizing the obstruction to the blood-flow within the muscle the observations of Heilemann[3] prove very suggestive. This author studied the circulation in the musculus submaxillaris of the frog under

[1] SADLER, W.: Arbeiten aus dem physiologischen Institute zu Leipzig, 1869, pp. 77–100.

[2] GASKELL, W. H.: Arbeiten aus dem physiologischen Institute zu Leipzig, 1877, pp. 45–88.

[3] HEILEMANN, H.: Archiv für Anatomie und Physiologie, 1902, pp. 45–53.

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the microscope. On tetanizing, or on stimulating this muscle by single induction shocks, he found that the flow was greatly retarded in those fine anastomosing venules which pass between the muscular fibres and parallel to them. Even a complete cessation of flow was observed at times, and if a strong tetanic current was used, an oscillating motion and even a backward movement of the column of blood resulted.

Differences in the force of the muscular contraction do not alter the general character of the curve. This statement is illustrated by

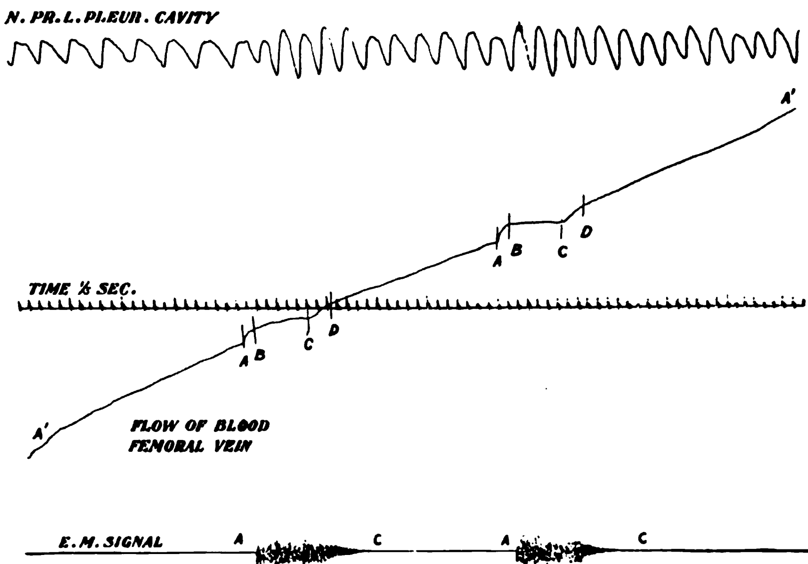


FIGURE 2. — Two-thirds the original size. Two successive variations showing different amplitude (tetanic muscular contraction).

Fig. 2. In this case two brief tetanic contractions of different strength were produced in quick succession. The sciatic nerve was stimulated first with a current of medium strength (distance of coils, 15 cm.) and subsequently with a strong current (distance of coils, 7 cm.)

We observe immediately that, although the details of the curve are the same in both cases, the changes in the blood-flow are more decisive during the strong muscular contraction. The rise of the curve on making the current (at *a*) is more sudden, steeper, and higher in the latter instance; which indicates that the volume of blood pro-

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pelled during the shortening of the strongly contracted muscle is proportionately greater.

The maximal shortening having been reached (at *b*), the curve inclines more strongly toward the abscissa in the latter case; the blood-flow is therefore more effectively retarded by the strongly contracted muscle (*b* to *c*). Whether a cessation of flow can be produced by a proportionate increase in the strength of the stimulation could not be determined with accuracy, because under these conditions the muscular movements were so forcible that the stromuhr was shifted out

TABLE XI.

THE CHANGES IN THE BLOOD-FLOW DURING A TETANIC MUSCULAR CONTRACTION.

| Taken from exp. | Phase. | Nerves stimulated. | Strength of current. Dist. of coils. cm. | Duration of stimulation in seconds. | Normal blood-flow. c.c. per sec. | Flow during period of musc. shortening. c.c. per sec. | Flow during tetanic state of muscle. c.c. per sec. | Flow after musc. relaxation. c.c. per sec. |
|---|---|---|---|---|---|---|---|---|
| 1 | 18 | sciatic | 15 | 3.5 | 1.08 | 2.7 | 0.63 | 1.25 |
| | 19 | " | 7 | 8.1 | " | 4.0 | 0.40 | 1.30 |
| 2 | 17 | " | 16 | 7.0 | 0.77 | 1.8 | 0.60 | 0.77 |
| | 18 | " | 10 | 7.3 | " | 2.0 | 0.52 | 0.90 |
| 3 | 21 | " | 15 | 5.1 | 0.63 | 1.4 | 0.45 | 0.67 |
| 4 | 16 | " | 15 | 10.3 | 0.73 | 1.1 | 0.65 | 1.0 |
| | 18 | " | 8 | 5.2 | " | 2.5 | 0.39 | 1.0+ |
| 6 | 10 | " | 13 | 6.5 | 1.06 | 2.3 | 0.70 | 1.06 |
| | 12 | " | 7 | 4.1 | " | 3.0 | 0.30 | 1.06+ |
| 7 | 11 | sciatic obturator | 12 | 8.9 | 0.55 | 1.7 | 0.48 | 0.60 |
| 9 | 5 | " | 6 | 3.7 | 0.50 | 2.9 | 0.18 | 0.80 |

of its normal position. The strongest stimulus applied was: distance of coils, 6 cm.; two dry cells.

The after-effect of the tetanic contraction, consisting in the brief rise above the normal value of the blood-flow (*c* to *d*), was generally more conspicuous after the application of a strong current. In the curve now under consideration this period of increased flow is well marked in both instances, but a decided quantitative difference is not evident.

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To show in a general way the pronounced differences in the blood-volume during the three principal phases of a tetanic muscular contraction, a number of these variations have been calculated, as closely as this is possible, in terms of cubic centimetres per second. The periods measured are marked in the preceding figures by the letters *a* to *b*, *b* to *c* and *c* to *d*.

Table XI also contains four instances in which the weak stimulation was followed by a stronger stimulation. The greater prominence of the changes resulting under these conditions (see Fig. 2) is clearly betrayed by these quantitative determinations.

The pressure-changes in the femoral vein, occurring during the tetanic contraction of the muscles of the posterior extremity, were recorded in most of the above experiments by means of a Hürthle's membrane-manometer (venous). This instrument, as stated before, recorded the pressure peripherally to the stromuhr.

On tetanizing the muscles the pressure quickly rose some millimetres above the normal value. At about point *b* of Figs. 1 and 2 the pressure decreased almost as rapidly as it had risen and kept subsequently very close to zero, falling generally even below the abscissa during the strong tetanic contraction. After the relaxation of the muscles the pressure remained slightly above normal for some time. If the tetanization was continued for a longer time, the pressure began to rise even before the break of the current.

The pressure-changes were also recorded in several separate experiments by means of a soda-manometer (sodium carbonate solution, specific gravity, 1.080) connected with the femoral vein by means of a T tube. A float was not used, the values being obtained by reading. The sciatic nerve was stimulated.

As is shown in Table XII, the pressure rapidly rose two to five mm. Hg above normal during the muscular shortening (*a* to *b*). The end of this period having been reached (at *b*), the pressure again decreased almost as quickly, assuming a value slightly below normal during the first part of the tetanization. Subsequently it gradually rose, remaining slightly elevated for a short time after the relaxation.

The only difference in the results of the former and latter methods consists therefore in the height of the pressure during the second period, the period of decreased flow (*b* to *c*). The soda-manometer indicated that the pressure does not drop to zero, but remains a few millimetres above the abscissa, which, it seems to me, is the correct result.

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Table XII, shows, moreover, that a stronger tetanization causes a greater rise in pressure during the period of great flow, muscular shortening (*a* to *b*). We have seen previously that under these conditions the blood-volume during the second period (*b* to *c*) is even more highly reduced; however, a correspondingly greater fall in pressure during this period could not be ascertained definitely, because the differences were so very slight.

TABLE XII.

THE CHANGES IN PRESSURE IN THE FEMORAL VEIN DURING A TETANIC MUSCULAR CONTRACTION.

(The corresponding values in mm. Hg are placed in brackets.)

| Experiment. | Wt. of dog. Kilos. | Duration of contr. in sec. | Strength of stimulus. Dist. of coils. cm. | Normal pressure. Mm. sod. carbonate. (Mm. Hg.) | Pressure at end of period of muscular shortening. Mm. sod. carbonate. (Mm. Hg.) | Pressure during contr. state of muscle. Mm. sod. carbonate. (Mm. Hg.) | Pressure at relaxation of muscle. Mm. sod. carbonate. (Mm. Hg.) |
|---|---|---|---|---|---|---|---|
| 1 | 13.0 | 7 | 15 | 78[6.2] | 100[7.9] | 70[5 5] | 78+[6 2+] |
| 2 | 11.5 | 5 | 10 | 98[7.7] | 140[11.1] | 88[7.0] | 110[8 7] |
| 3 | 14.0 | 11 | 10 | 65[5.1] | 112[8.9] | 55[4.3] | 78[6.2] |
| 4 | 16.5 | 8 | 8 | 72[5.7] | 132[10.5] | 60[4.7] | 80[6.3] |
| 5 | 16.5 | 7 | 5 | 72[5.7] | 145[11.5] | 60[4.7] | 80[6.3] |
| 6 | 16.0 | 10 | 12 | 62[4.9] | 85[6.7] | 58[4.6] | 62+[4.9+] |

**Single induced current.** — If single induction shocks are used, the variations in the blood-flow accompanying the muscular twitches, show a somewhat different outline.

Fig. 3 may serve to illustrate the general character of the variations obtained under these conditions. The sciatic nerve was stimulated in this instance with a current of medium strength, distance of coils, 10 cm.

Both the make (*a*) and the break (*c*) of the current are followed by a considerable increase in the blood-volume which continues during the entire periods of rising energy of the muscles (*a* to *b* and *c* to *d*). Furthermore, this rise is directly proportionate to the strength of the stimulus, and therefore also to the force of the muscular contraction. The stronger the twitch, the more conspicuous is the increase in the venous blood-flow.

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During the second period, *i. e.*, while the current is passing, the blood-volume is not reduced as during the tetanization of the muscles, but remains normal. The blood-flow resumes its normal value immediately after the rise occurring during the make-contraction (*a* to *b*), and retains it until the break-twitch causes another period of increased flow (*c* to *d*). Subsequent to the latter rise the blood-flow immediately returns to normal.

The fact that the blood-volume is not reduced while the current passes, greatly influences the total quantity of blood propelled in a

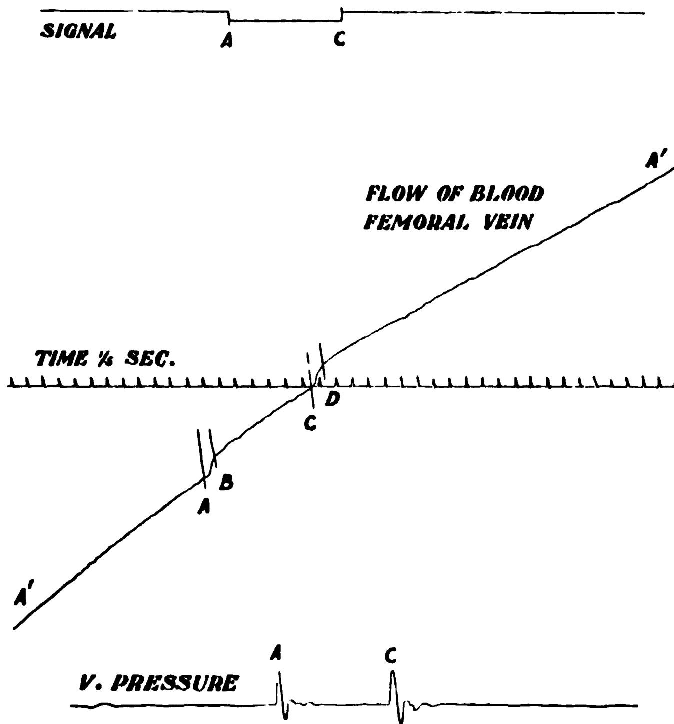


FIGURE 3.—Two-thirds the original size. Variation in venous blood-flow accompanying single muscular twitches (*a*, make, *c*, break of current).

given time. To illustrate: supposing the stromuhr has written three successive phases of the same length. In the first phase only the normal blood-flow is recorded. During the second phase a strong tetanic contraction has been produced, while during the third a single induced current of equal strength and duration has been applied. In

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the second case it will be found that the total quantity of blood pro pelled during the phase is considerably less than the normal volume. The decrease will be the greater, the longer the duration of the tetanization and the stronger the current. Even if the blood-flow is greatly increased by the muscular shortening, the reduction in the blood-volume during the contracted state of the muscle far outweighs the former effect. In the third case, on the other hand, the total quantity will be greater than normal, the increase being proportionate to the volume of blood propelled during the rising periods of the muscular twitches.

The greatest increase in the venous blood-flow is therefore produced by a series of single muscular twitches. The same result can be obtained by successive tetanic contractions, but their duration must be very brief, so that only the quantitative effect of the period of muscular shortening can become evident. If continued for a long time, the retardation of the flow during the tetanic state of the muscle will naturally cause a decided decrease in the total quantity of blood propelled.

## Summary.

1. The value of the blood-flow measured in nine dogs varied from 0.50 c.c. per second to 1.20 c.c. per second. The average flow was 0.85 c.c. per second; average weight of dog, 14.2 kilos.

2. The velocity of the blood-stream varied from 48.5 mm. per second to 74.7 mm. per second; average velocity, 61.6 mm. per second.

3. Compression of the femoral artery caused a reduction in the blood-volume of from 63 to 90 per cent; average decrease, 75 per cent.

4. Section of the nerves innervating the posterior extremity was followed by an increase in the blood-volume of from 2.6 to 3.1 times the normal value; average increase, 2.8 times.

5. The variations in the venous blood-flow accompanying a tetanic muscular contraction may be divided into three periods: —

1. Period of great flow, synchronous with the muscular shortening.

2. Period of slight flow, continuing during the contracted state of the muscle.

3. Short period of increased flow, following the relaxation of the muscle.

Differences in the force of the muscular contraction do not alter the

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general character of the variation, but only cause changes in the amplitude of its various details.

6. If single induction shocks are used, a great increase in the flow results during the periods of rising energy of the muscle. Between the twitches the blood-flow resumes its normal value.

7. The venous pressure changes during a tetanic contraction in a corresponding manner. There is a quick rise during the muscular shortening and an almost equally rapid fall after the maximal contraction has been reached. During the first part of the tetanization, the pressure remains slightly below normal, while during its latter part the pressure gradually rises and continues slightly above normal for a short time after the relaxation of the muscle.

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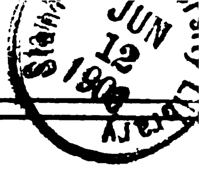



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# THE INFLUENCE OF FORMALDEHYDE ON THE ACTION OF CERTAIN LAKING AGENTS AND ON COAGULATION OF BLOOD.

BY CHARLES CLAUDE GUTHRIE.

[*From the Physiological Laboratories of the University of Chicago and Western Reserve University.*]

IT has been shown by Dr. G. N. Stewart,[1] that where formaldehyde is added to blood in sufficient amount to cause ultimately complete fixation of the corpuscles, laking can be brought about by various hæmolytic agents, including saponin, for a certain period after the addition of formaldehyde. The length of this period and the completeness of the laking depend, of course, on the amount of formaldehyde added, as well as on the strength of the laking agent. At his suggestion I undertook a series of experiments to determine for how long a period blood-corpuscles still remain susceptible to the action of haemolytic agents after the addition of smaller amounts of formaldehyde than were employed by him, and in particular to determine whether such quantities of formaldehyde as were just necessary to prevent putrefaction of the blood would exert a restraining influence on the action of laking agents. If this question should be answered in the affirmative, it seemed not impossible that a feasible method of preserving blood for hæmolytic tests, at least for several days, might be arrived at, *e.g.* by mixing blood with formaldehyde and potassium oxalate, the former being for the purpose of restraining putrefaction, and the latter, coagulation, — a point of some importance, especially in connection with the investigation of biological hæmolysins. The fact that formaldehyde fixation of hæmoglobin does not completely prevent the corpuscles from being acted on by some hæmolytic agents (*e.g.* saponin), since their permeability for electrolytes is still increased by them, indicates that certain constituents of corpuscles which are related to the laking process are not fixed by formaldehyde. The investigation of the relation between formaldehyde fixation and laking seems, therefore, calculated to throw light on

[1] STEWART: Journal of physiology, 1901, xxvi, p. 470.



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the process of hæmolysis. I had already been making some investigations on the effects of intravenous injection of formaldehyde, in the course of which certain phenomena were observed which, on being reported to Dr. Stewart, led him to suggest that I should combine with the experiments on the influence of formaldehyde on laking agents, a series of experiments on the action of that substance on coagulation of the blood. This accounts for the manner in which the results are reported in this note. I hope to have an opportunity to return to this subject.

The method of procedure was as follows: Test-tubes were graduated in 10 c.c. by means of narrow strips of gummed paper, labelled and arranged in series in racks. The reagent to be mixed with the blood was added by means of a pipette, graduated in 0.01 c.c., immediately before adding the blood. The animal which was to furnish the blood was anæsthetized with A.C.E. mixture, and a vaselined glass cannula, connected with a piece of vaselined rubber tubing 10 cm. long, was tied in one of the common carotid arteries. The blood was then allowed to flow into the test-tubes up to the graduate mark or as near it as possible, the flow being regulated by pressure on the rubber tube with the fingers. The tube was then closed with the thumb, and vigorously shaken to thoroughly mix the contents. The time required to fill a tube and mix the contents was very short, the average of a large number being eleven seconds. All tubes were equally shaken, so that the contents of all might be equally agitated or mixed, as the case might be. The formaldehyde solutions were freshly prepared each time, excepting in the cases noted where solutions several weeks old were used for the purpose of determining their activity. Schering's formalin, which has been shown by Dr. Torald Sollmann[1] to contain 39.9 per cent formaldehyde, was added to 0.9 per cent NaCl in sufficient quantity to make the solutions of the desired strengths. The potassium oxalate solution was prepared by adding the salt to 0.9 per cent NaCl solution in the proper proportion to make 1 per cent solutions.

The following are the results obtained:

1. Coagulation is retarded in blood drawn into solutions of formaldehyde in proportion to the amount of formaldehyde present in the resulting mixture. (Table II.)

2. Formaldehyde in sufficient amount prevents coagulation indefinitely, but the amount necessary to do this varies with different animals of the same species. In eight experiments, seven dogs and one rabbit being used, the amount necessary to prevent coagulation

[1] SOLLMANN: This journal, 1902, vii, footnote, p. 225.

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ranged from one part of formaldehyde to 66.6 parts of blood to 1 to 400, and gave an average of 1 to 185.5. That varying amounts are necessary to prevent coagulation in different animals may of course be due in part to variations in the number of corpuscles per cubic centimetre of blood.

In making such observations it is necessary to take into account certain possible sources of error. The more common ones are mentioned below:

If to tubes containing equal amounts of formaldehyde, blood be added at intervals, it will be seen that the more blood-clot the blood comes in contact with before flowing into the tube, the shorter the coagulation time will be. This is shown by Table II, Tubes 4, 17, 26, 35, and 47, as well as by a number of other similar experiments.

By reference to Tubes 7, 8, 19, 28, 37, 49, and 57, it will be observed that all but one of them (37) showed coagulation in greater or less degree. The coagulation time gradually became longer until this tube was reached, when it began to shorten and continued to become shorter to the end of the series, Tube 57 coagulating in a shorter space of time than the first (7). The reason of this variation in the amount necessary to prevent coagulation in different samples of blood from the same animal, is not quite clear. It may be due either to the formaldehyde retarding or preventing coagulation by restraining the formation of fibrin ferment, or by a direct action on already formed fibrin factors. The former explanation seems the more likely, as blood drawn through a tube containing fresh blood clot must contain more fibrin ferment than blood drawn through a clean tube. My experiments on this point are still incomplete.

The coagulation time of normal blood is very materially shortened, as is well known, by drawing it through a tube contaminated with fresh blood clot. This is shown by Tubes 1, 10, 20, 30, 40, 50, 58, and 59. In this experiment coagulation was not hastened merely by loss of blood, to any appreciable extent, as shown by Tube 60, which only differed from the tubes enumerated above, in being drawn from the opposite common carotid and through a perfectly clean tube. It will be seen by comparing this tube with 58 and 59, that it required almost four times as long to clot, and slightly longer than 1. It has been shown by Arthus[1] that coagulation is accelerated by bleeding. It appears, however, that this is not so powerful a factor as contact with clotted blood.

Asphyxia is another important factor in retarding coagulation, which is worth while bearing in mind, though if the blood be drawn through a cannula containing clot, the effect is to a great extent lost. This is shown in Tube

[1] ARTHUS: Journal de physiologie et de pathologie générale, 1902, iv, p. 283.

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61. In one of my experiments, blood drawn by puncturing the right ventricle and allowing the blood to spurt into a test-tube, ten to fifteen minutes after the arterial pressure had fallen to zero (of course, plus residual pressure), from asphyxia, coagulation was delayed for some hours, the corpuscles having settled before it occurred.

That the dilution of blood is not sufficient to account for the delays noted on addition of solutions of formaldehyde, is shown by comparing the series 29, 31, 32, and 33 with either of the series occurring just prior or subsequent to it. The only difference in this and the two series mentioned is that they contain formaldehyde in the amounts stated in the table, while it consists of mixtures of 0.9 per cent NaCl and blood only. In the NaCl tubes there is only a slight delay which is accounted for by the dilution.

3. Amounts of formaldehyde too small to prevent coagulation, prevent the clot from advancing beyond a delicate jelly-like stage, for a period proportionate to the amount of formaldehyde present. With amounts below 1 to 1400, contraction very gradually occurs, though 1 to 50,000 markedly delays contraction and prolongs the jelly stage. On washing normal clots and jelly clots, and comparing the resulting insoluble substances, the normal clot shows for the most part dense masses of fibrin, the fibres of which are easily seen under the microscope, while the formaldehyde clots show brownish granular masses or less gelatinous residues, having no gross or microscopic characters of true fibrin. Treated with Löffler's methylene blue and examined microscopically, they are seen to be made up of irregular granular masses and leucocytes, and red corpuscles more or less laked.

4. The onset of spontaneous laking[1] at room temperature is not markedly delayed by small quantities of formaldehyde, but, once begun, it proceeds much more slowly than in normal blood. This is shown by comparing Tubes 1, 10, 20, 30, 40, 50, 58, 59, 60, and 61, with the tubes containing formaldehyde. Larger quantities up to a certain point greatly hasten laking. The amount necessary to fix the corpuscles with practically no laking varies with different specimens of blood, owing probably to the variations in the number of corpuscles per c.c. of blood. As a rule, though not always, the amount of formaldehyde necessary to prevent coagulation also largely prevents laking, the corpuscles settling to the bottom of the vessel and gradually assuming a chocolate-brown color. Corpuscles treated with for-

[1] It has been shown, *e. g.* by Dr. G. N. STEWART (Journal of physiology, 1899, xxiv, p. 228), that sterile blood lakes when it is allowed to stand.

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maldehyde in proportions up to 1 part of blood to 4 parts of 1 per cent formaldehyde for as much as forty-eight hours, and then washed free from plasma with 0.9 per cent NaCl solution, are slowly but markedly laked by water and solutions of sapotoxin.

6. The influence of formaldehyde on laking by foreign serum was determined in the following manner. (Table I.)

Approximately two thirds of the total amount of blood was drawn from a dog, and allowed to clot. 1 per cent solution of formaldehyde in 0.9 per cent NaCl solution was then injected into a vein in about the proportion of 1 to 1500 of the remaining blood. Five minutes after the completion of the injection, the remainder of the blood was drawn and allowed to clot. Rabbit's blood was then drawn and defibrinated. All the specimens were then placed on ice and allowed to remain for sixteen hours, when they were removed and the action of formaldehyde studied.

Rabbit's blood and dog's serum were treated separately with various proportions of formaldehyde, for ten minutes, and then mixed with equal amounts of blood or serum, as the case might be, formaldehyde rabbit's blood being mixed with untreated dog's serum and vice versa. The mixture of blood and serum was allowed to stand ten minutes, and was then centrifugalized five minutes, and the supernatant serum examined.

(*a*) Rabbit's blood plus formaldehyde, in the proportion of 1 to 1000, is moderately laked by dog's serum in the time specified. Larger amounts prevent laking. Blood treated with amounts too small to prevent laking, are laked in proportion to the amount present.

(*b*) Dog's serum plus formaldehyde in proportion of 1 to 2000 or less, still retains its power of laking rabbit's corpuscles, the amount of laking depending on the proportion of formaldehyde added. Formaldehyde, in the proportion of 1 to 1000 or above, restrains all laking for the time specified. It will be seen that a smaller amount of formaldehyde will restrain laking when it is added to dog's serum previous to the mixture of the serum and the rabbit's blood, than when it is first added to the rabbit's blood.

(*c*) Serum from blood drawn from the dog after injection of formaldehyde, in the proportion of about 1 to 1500, lakes rabbit's corpuscles as energetically as serum from blood drawn before the injection.

(*d*) Mixture of formaldehyde bloods and sera, containing more than enough formaldehyde to prevent biological laking, are rapidly and strongly laked by water or sapotoxin solution in 0.9 per cent NaCl solution.

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7. Spectroscopically no obvious change is discoverable in the hæmoglobin when formaldehyde is added to blood in the proportion of 1 to 166, after a period of four days, or after the action of smaller amounts for longer periods of time. With amounts adequate to prevent contraction of the clot and putrefaction, the blood shows no obvious spectroscopic change even after the lapse of weeks.

8. Blood can be well preserved for a number of days at room temperature by adding potassium oxalate in sufficient amount to prevent coagulation, and formaldehyde in the proportion of 1 to 1000 to 500 or more, to retard laking and putrefaction. In the series of such tubes given in Table II, Tube 53 showed only a small amount of laking after being kept in a warm room for ten days. On the subsequent addition of water, rapid and complete laking occurred.

The amount of potassium oxalate necessary to add to blood was found to vary considerably in different dogs. In four experiments, the largest amount required was 1 part to 300 of blood; the smallest, 1 part to 700; the average amount was 1 part to 534. Arthus recommends 3 parts to 1000.

Blood to which sufficient potassium oxalate solution has been added to prevent coagulation, after standing some days at room temperature, will frequently be found to have undergone coagulation, as has been pointed out by Schäfer.[1] In no case observed by me has this solidification occurred under five days from the time of drawing the blood.

In tubes containing a sufficient amount of formaldehyde to prevent clotting, and in tubes containing a sufficient amount of potassium oxalate to prevent clotting, and to which small amounts of formaldehyde have been added, brownish, and more or less gelatinous precipitates, which float or remain suspended near the surface of the liquid, appear in periods of time, ranging from a few hours to several days (depending on the amount of formaldehyde present).

[1] SCHÄFER: Text-book of physiology, i, p. 169, footnote 7.

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TABLE I.

| Tube. | 1% formaldehyde. | Blood q. s. | Serum q. s. | Proportion of formaldehyde to blood or serum. | Time. Formaldehyde acted. | Time. Serum acted. | Result. |
|---|---|---|---|---|---|---|---|
| A. 1 | .. | 1.0 | 2.0 | .. | .. | 10 min. | Very strongly laked. |
| 2 | 0.1 | 1.0 | 2.0 | 1-1000 | 10 min. | 10 " | Less strongly laked. |
| 3 | 0.2 | 1.0 | 2.0 | 1-500 | 10 " | 10 " | Not laked. |
| 4 | 0.3 | 1.0 | 2.0 | 1-333 | 10 " | 10 " | " " |
| 5 | 0.4 | 1.0 | 2.0 | 1-250 | 10 " | 10 " | " " |
| B. 15 | 0.05 | 2.0 | 1.0 | 1-2000 | 10 " | 16 " | Slightly laked. |
| 16 | 0.10 | 2.0 | 1.0 | 1-1000 | 10 " | 10 " | Not laked. |
| 17 | 0.15 | 2.0 | 1.0 | 1-666 | 10 " | 10 " | " " |
| 19 | form. 0.5% 0.10 | 2.0 | 1.0 | 1-2000 | 10 " | 10 " | Strongly laked. |
| 20 | 0.05 | 2.0 | 1.0 | 1-4000 | 10 " | 10 " | More strongly laked. |
| 21 | Formaldehyde injection into veins in proportion 1-1500 of the blood. | | | | | | |
| | | 2.0 | 1.0 | .. | .. | 10 " | As strongly laked as 1. |

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TABLE II.

| Tube No. | Reagents. | Blood q. s. | Dilution of formal-dehyde to blood, 1 to | Time blood drawn after opening artery. | Time required to clot. | After 21 hours. | After 68 hours. | Time of laking. |
|---|---|---|---|---|---|---|---|---|
| | | c.c. | | secs. | secs. | | | hours. |
| 1 | . . . . . . . . . | 10.00 | .. | 0 | 110 | No laking, dark clot, clear serum | Laked, putrid | 48 to 68. |
| 2 | 2 c.c. 1% form. (fresh) | 10.25 | 512.5 | 10 | 290 | Red jelly clot; no serum or laking | No serum | 21 to 48. |
| 3 | 0.4 c.c. 5% form. (fresh) | 10.20 | 510 | 20 | 265 | Same as 2 only slightly darker | No serum | Less than 21. |
| 4 | 4 c.c. 1% form. (fresh) | 11.10 | 277.5 | 30 | 300 | Same as 3 only slightly darker; laked | No serum | " " |
| 5 | 0.8 c.c. 5% form. (fresh) | 10.50 | 262.5 | 40 | 230 | Same as 4 only much darker; laked | No serum | " " |
| 6 | 6 c.c. 1% form. (fresh) | 11.00 | 183.3 | 50 | 520 | Darker than 5; laked; jelly | Drop red serum | " " |
| 7 | 1.2 c.c. 5% form. (fresh) | 10.75 | 179.2 | 60 | 330 | Laked; darker than 6; jelly | No serum | " " |
| 8 | 8 c.c. 1% form. (fresh) | 11.00 | 137.5 | 70 | 1520 | Part clot; strongly laked; very dark | Much serum | " " |
| 9 | 1.6 c.c. 5% form. (fresh) | 10.60 | 132.5 | 80 | 2840 | More clot than 8; strongly laked; dark as 8 | Soft jelly | " " |
| 10 | . . . . . . . . . | 10.10 | .. | 85 | 70 | Same as 1; no laking | Laked, putrid | 21 to 48. |
| 16 | 2 c.c. 1% form. (fresh) | 10.10 | 505 | 124 | 221 | Same as 4 | No serum | Less than 21. |
| 17 | 4 c.c. 1% form. (fresh) | 10.00 | 250 | 135 | 195 | Same as 6 | No serum | " " |
| 18 | 6 c.c. 1% form. (fresh) | 10.20 | 170 | 141 | 339 | Jelly; color same as 8 | No serum | " " |
| 19 | 8 c.c. 1% form. (fresh) | 10.75 | 134.3 | 147 | 453 | Soft jelly; same as 8 in color | Soft jelly | " " |
| 20 | . . . . . . . . . | 11.50 | .. | 150 | 75 | Same as 10; no laking | Moderately laked, slightly putrid | 48 to 68. |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 21 | 2 c.c. 1% form. (old) | 10.00 | 500 | 159 | 100 | Same as 16 only slightly darker | No serum | Less than 21. |
| 22 | 4 c.c. 1% form. (old) | 10.20 | 255 | 168 | 163 | Same as 17 only slightly darker | No serum | " " |
| 23 | 6 c.c. 1% form. (old) | 10.30 | 171 | 177 | 530 | Same in color and consistency as 19 | Same as 19 | " " |
| 24 | 8 c.c. 1% form. (old) | 10.40 | 130 | 186 | 1303 | Same consistency as 8; slightly darker | More fluid than 23 | " " |
| 25 | 2 c.c. 1% form. (fresh) | 10.00 | 500 | 195 | 132 | Same as 21 | No serum | " " |
| 26 | 4 c.c. 1% form. (fresh) | 10.20 | 255 | 204 | 195 | Same as 22 | No serum | " " |
| 27 | 6 c.c. 1% form. (fresh) | 10.30 | 171 | 213 | 303 | Same as 23 only more solid | Soft jelly | " " |
| 28 | 8 c.c. 1% form. (fresh) | 11.00 | 127 | 222 | 1356 | Same as 24 only slightly more solid | Part clot | " " |
| 29 | 2 c.c. 0.9% NaCl | 10.20 | .. | 231 | 55 | Same as 30 only slightly more serum; no laking | Slight laking and putrefying | 48 to 68. |
| 30 | . . . . . . . . . | 10.00 | .. | 240 | 45 | Same as 1; no laking | Stronger laked and putrid | " " |
| 31 | 4 c.c. 0.9% NaCl | 11.30 | .. | 249 | 115 | Same as 29 only more serum; no laking | Stronger laked and more putrid | " " |
| 32 | 6 c.c. 0.9% NaCl | 10.75 | .. | 258 | 221 | Same as 31 only more serum; no laking | Stronger laked and putrid | " " |
| 33 | 8 c.c. 0.9% NaCl | 10.00 | .. | 267 | 112 | Same as 32 only more serum; no laking | More laked and putrid | " " |
| 34 | 2 c.c. 1% form. (fresh) | 10.00 | 500 | 276 | 74 | Same as 25 | No serum | Less than 21. |
| 35 | 4 c.c. 1% form. (fresh) | 10.70 | 267 | 285 | 126 | Same as 26 | No serum | " " |
| 36 | 6 c.c. 1% form. (fresh) | 11.00 | 183 | 294 | 415 | Same as 27 | Soft jelly | " " |
| 37 | 8 c.c. 1% form. (fresh) | 10.30 | 128 | 303 | .. | Same as 28 in color; slight or no clot | . . . . . . . . | " " |
| 38 | {0.025 c.c. 1% form. (fresh)<br>{3 c.c. 1% potass. oxal. | 10.10 | 40400 | 312 | .. | Red corpuscles; clear plasma | Very strongly laked and putrid | 48 to 68. |
| 39 | {0.05 c.c. 1% form. (fresh)<br>{3 c.c. 1% potass. oxal. | 11.00 | 21200 | 321 | .. | Red corpuscles; clear plasma | Less laked and putrid than 38 | " " |

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TABLE II — (*continued*).

| Tube No. | Reagents. | Blood q. s. | Dilution 1 to | Time blood drawn after opening artery. | Time required to clot. | After 21 hours. | After 68 hours. | Time of laking. |
|---|---|---|---|---|---|---|---|---|
| | | c.c. | | secs. | secs. | | | hours. |
| 40 | . . . . . . . . . | 10.60 | .. | 330 | 90 | Same as 1; no laking | Strongly laked and putrid | 48 to 68. |
| 41 | 0.1 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.75 | 10750 | 340 | .. | Same as 38 only slightly redder corpuscles and laked | Slightly less laked than 39 | Less than 48. |
| 42 | 0.2 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.20 | 5400 | 350 | .. | Same as 41 only slightly redder corpuscles and slightly laked | Less laked than 41 | " " |
| 43 | 0.4 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.00 | 2500 | 360 | .. | Same as 42 slightly redder and small amt. prec. in plasma | Less laked than 42; reddest pot. ox. tube | 48 to 68. |
| 44 | 0.6 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.40 | 1733 | 370 | .. | Slightly darker than 43 and more brown prec. in plasma | Slightly more laked than 43 | Less than 48. |
| 45 | 0.7 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.60 | 1714 | 380 | .. | Slightly darker than 44 and much more prec. in plasma | Slightly more laked than 44 | " " |
| 46 | 2 c.c. 1% form. (fresh) | 10.00 | 500 | 390 | 105 | Same as 34 | No serum | Less than 21. |
| 47 | 4 c.c. 1% form. (fresh) | 10.50 | 262 | 400 | 112 | Same as 35 | No serum | " " |
| 48 | 6 c.c. 1% form. (fresh) | 10.40 | 173 | 410 | 374 | Same as 36 or slightly softer | Some serum | " " |
| 49 | 8 c.c. 1% form. (fresh) | 11.10 | 138 | 420 | 187 | Same as 37 only more solid | More serum than 48 | " " |
| 50 | . . . . . . . . . | 10.00 | .. | 430 | 45 | Same as 1; no laking | Laked | 48 to 68. |
| 51 | 0.8 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.50 | 1312 | 411 | 336 | Color same as 43, soft jelly (?) and small amt. serum; sl. laking | Less laked than 43 | " " |
| 52 | 0.9 c.c. 1% form. (fresh) 3 c.c. 1% potass. oxal. | 10.75 | 1194 | 452 | .. | Color same as 51; more plasma and brown prec. than 45 | Slightly more laked than 51 | " " |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 53 | {1 c.c. 1% form. (fresh) / 3 c.c. 1% potass. oxal.} | 11.25 | 1125 | 463 | 1685(?) | Same as 52 only more serum (?); no laking | Sl. more laked than 51 and less than 52 | |
| 54 | 2 c.c. 1% form. (fresh) | 10.20 | 510 | 474 | 111 | Same as 46 | No serum | Less than 21. |
| 55 | 4 c.c. 1% form. (fresh) | 10.50 | 262 | 485 | 457 | Same as 47 | Some serum | " " |
| 56 | 6 c.c. 1% form. (fresh) | 10.50 | 175 | 496 | 99 | Same as 48 only more solid | Soft jelly | " " |
| 57 | 8 c.c. 1% form. (fresh) | 10.75 | 133 | 507 | 118 | Same as 49 only more solid | More serum than 56 | " " |
| 58 | . . . . . . . . . | 11.00 | .. | 518 | 30 | Same as 1; no laking | Laked | 48 to 68. |
| 59 | . . . . . . . . . | 10.00 | .. | 529 | 35 | Same as 58 | Laked | " " |
| 60 | . . . . . . . . . | 10.10 | .. | 540 | 125 | Most serum of any similar tube; no laking | Slight or no laking | " " |
| 61 | . . . . . . . . . | 7.00 | .. | 1010 | 60 | Less serum than any "normal" tube; no laking | Strongly laked | Less than 48. |

NOTE: Tubes 58, 59, and 60 differ only in that the blood in the two former was drawn through a tube containing blood clot, while in the latter, the blood was drawn from the corresponding artery through a clean tube.

Tube 61 differs from the ones just mentioned in that the blood was drawn about 4 mins. after the pressure had fallen to zero from asphyxia. It was drawn through the tube containing the blood clot.

Tubes 51 and 53 showed no true coagulation but a marked viscidity.

The terms *serum* and *plasma* are rather loosely used, owing to the difficulty of ascertaining definitely whether or not slight coagulation occurred in some of the tubes.

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# VENOUS PRESSURES.

By RUSSELL BURTON-OPITZ.

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## Normal Venous Pressure.

IN this investigation dogs were used exclusively. They were placed upon their backs with the posterior extremities slightly flexed and abducted. The anterior extremities were fastened against the sides of the chest and forced downward so that, by slightly depressing the shoulder, more easy access could be had to the lower portion of the external jugular vein. The head and neck were brought as nearly as possible to the horizontal plane of the body.

All the animals experimented on were anæsthetized with ether. Morphine was given only to the larger animals, and in comparatively small doses. During the recording of the pressures, deep narcosis was avoided.

The determinations were made with manometers (4.5 mm. tubing) filled with a concentrated sodium carbonate solution of the specific gravity 1,088. They were connected with the veins by means of T tubes, the horizontal branches of which were very short and of the same diameter as the vein. A stop-cock, interposed between the T tube and the manometer, served to minimize the excursions of the liquid. It need hardly be mentioned that the pressure, recorded in this manner, is the average lateral pressure.

The level of the liquid in the different manometers having been brought to that of the corresponding vein, all the clamps were quickly



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removed and the pressures read simultaneously during the next three to five minutes. The average reading was subsequently derived from these data.

The pressure was determined in the following veins:[1]

1. Left facial vein.
2. Left and right external jugular veins.
3. Superior vena cava, distal portion.
4. Superior vena cava, in the neighborhood of the right auricle.
5. Left and right femoral veins.
6. Left saphenous vein.
7. Right brachial vein.

The left facial vein was connected with the manometer peripherally to the superficial laryngeal lymphatic glands, almost opposite the angle of the jaw.

The pressure in the external jugular vein was recorded at the point where this vessel leaves the surface and enters deeply into the groove between the neck and the shoulder. A horizontal line drawn through the tip of the manubrium marks the point of insertion of the T tube. In some animals, however, it was necessary to place the tube two or three centimetres above this level, because the shoulders were so prominent that a correct adjustment of the manometer was impossible.

In many of the experiments a catheter was introduced into the right external jugular vein by means of which the pressure was registered in the distal and central portions of the superior vena cava.

For the sake of better orientation, the catheter was inserted diagonally at first. Having reached the superior vena cava, *i.e.*, a point central to the bifurcation of the subclavian veins, a rise in the pressure of the opposite jugular vein resulted, because the catheter interfered with the influx of blood from this vein. The catheter being subsequently brought into the axis of the superior vena cava by placing it against the side of the neck, the increase in pressure immediately disappeared.

The entrance to the right auricle was determined approximately by introducing the catheter, first, into the right ventricle, and subsequently withdrawing it a sufficient distance. If a small catheter is used, and the insertion is made in the axis of the superior vena cava, absolutely no change in the level of the liquid in the other manometers results.

[1] The nomenclature is the same as in ELLENBERGER and BAUM'S Anatomie des Hundes, pages 432 to 457.

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The T tube was inserted in the femoral vein midway between the groin and the orifice of the vena postica superior, about 3 cm. below the groin. In those cases in which the pressure was also recorded in the left saphenous vein, the femoral vein was connected with the manometer immediately below the groin, while the latter vein was secured close to the median edge of the gracilis muscle, near the tip of Scarpa's triangle.

The pressure in the brachial vein was obtained in but two experiments, because of the difficulty of correctly adjusting the manometer to this blood-vessel.

The distances between the different manometers were measured in almost all the animals for the purpose of calculating subsequently the average fall in pressure from the periphery to the centre of the circulatory system. Naturally, these measurements could be made only approximately (air-line measurements).

The respiratory movements were recorded by means of a tambour which communicated with the left pleural cavity. Although the variations in intrathoracic pressure are of great importance in an investigation of this kind, a detailed account need hardly be given. As the experimental conditions were in all cases as normal as possible, the changes in intrapleural pressure moved within normal limits. The greatest negative pressure prevailed in Experiment 15, the lowest in Experiment 7; in the former case it equalled 11 mm. Hg, in the latter instance 6 mm. Hg.

The results obtained from eighteen experiments are comprehensively arranged in Table I. To prevent confusion, and to have at the same time a more ready means for comparison, the pressure is given in millimetres of mercury. In transferring the values obtained with sodium carbonate solution, the following formula was employed:

$$H\ (\text{mm. Hg}) = \frac{H\ (Na_2CO_3\ \text{sol.}) \times \text{spec. grav. } (Na_2CO_3\ \text{sol.})}{\text{spec. grav. Hg}}$$

Table I shows very clearly that the pressure gradually decreases from the periphery toward the centre of the circulatory system. If the average value is taken, it is observed that the pressure in the left facial is 4.6 mm. Hg higher than in the left external jugular vein, in the vicinity of the chest. The manometers in these veins were on the average 150 mm. apart. The fall in pressure amounts, therefore, to 1.0 mm. Hg for every 32 mm. distance.

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TABLE I.

VENOUS PRESSURE IN MM. HG.[1]

(Ether-narcosis light in all cases. Respiration normal).

| Experiment. | Weight of dog. | Morphine. | Left facial vein. | Left external jugular vein. | Right external jugular vein. | Superior vena cava. | | Left femoral vein. | Right femoral vein. | Left saphenous vein. | Right brachial vein. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Distal port. | Central port. | | | | |
| | kilos. | grams. | | | | | | | | | |
| 1 | 13.5 | 0.08 | 5.1 | −0.48 | −0.4 | .. | .. | 5.8 | 6.1 | | |
| 2 | 11.5 | 0.08 | 4.6 | 0.16 | .. | .. | .. | 7.6 | .. | 8.8 | |
| 3 | 16.5 | 0.10 | 5.7 | 0.80 | .. | .. | .. | 5.2 | 5.3 | .. | 4.2 |
| 4 | 14.0 | 0.08 | 5.7 | −0.4 | .. | −1.2 | .. | .. | 5.4 | | |
| 5 | 16.5 | 0.10 | 6.0 | 2.8* | .. | 0.6 | .. | .. | 5.7 | | |
| 6 | 20.0 | 0.08 | 6.5 | 0.7 | .. | −2.4 | −4.4 | 5.6 | | | |
| 7 | 19.5 | 0.10 | 4.1 | −0.08 | .. | −1.6 | −2.9 | .. | 6.8 | | |
| 8 | 18.0 | 0.08 | 3.6 | 0.2 | .. | −2.0 | −2.8 | 4.4 | .. | 5.7 | |
| 9 | 16 5 | 0.08 | 4.0 | −0.8 | .. | −2.2 | −2.9 | 5.6 | | | |
| 10 | 17.5 | 0.08 | 5.6 | 0.7 | .. | −0.16 | −1.2 | 4.4 | 4.5 | | |
| 11 | 7.5 | 0.0 | .. | −0.4 | .. | −1.7 | −3.4 | 3.2 | | | |
| 12 | 17.0 | 0.12 | 5.2 | 2.0* | .. | −1.4 | .. | 5.0 | | | |
| 13 | 6.0 | 0.0 | .. | 0.2 | 0.16 | .. | .. | 5.6 | 5.6 | | |
| 14 | 6.5 | 0.0 | .. | −0.8 | .. | −2.4 | .. | 6.3 | | | |
| 15 | 24.5 | 0.15 | .. | 2.4* | .. | −0.4 | −2.0 | 6.2 | .. | 6.8 | |
| 16 | 9.0 | 0.0 | 5.4 | 1.2* | .. | 0.0 | −1.4 | 5.7 | | | |
| 17 | 7.0 | 0.0 | .. | 0.0 | −0.08 | .. | .. | 3.8 | 4.0 | .. | 3.7 |
| 18 | 13.0 | 0.08 | .. | −0.5 | .. | −1.7 | −4.8 | 6.4 | .. | 8.4 | |
| Av. value | 14.1 | .. | 5.12 | 0.52 | −0.08 | −1.38 | −2.96 | 5.39 | 5.42 | 7.42 | 3.9 |
| Highest v. | .. | .. | 6.5 | 2.8 | 0.16 | 0.6 | −1.2 | 7.6 | 6.8 | 8.8 | 4.2 |
| Lowest v. | .. | .. | 3.6 | −0.8 | −0.4 | −2.4 | −4.8 | 3.2 | 4.0 | 5.7 | 3.7 |
| Difference | .. | .. | 2.9 | 3.6 | 0.56 | 3.0 | 3.6 | 4.4 | 2.8 | 3.1 | 0.5 |

[1] In this connection mention must also be made of the determinations of the venous pressure in sheep made by JACOBSON and reported in the Archiv für Anatomie und Physiologie, 1867, page 226.

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In the larger dogs the distance between the manometers in the facial vein and in the central portion of the superior vena cava, near the entrance to the right auricle, measured approximately 290 mm. The fall in pressure between these respective points is 8.08 mm. Hg, or 1.0 mm. Hg for every 35 mm. distance.

The radius from this centre to the saphenous vein measured approximately 380 mm. The fall in pressure amounts to 10.38 mm. Hg. The average decrease is 1.0 mm. Hg for every 36 mm. distance.

The average fall in pressure from the left femoral vein to the entrance to the right auricle amounts to 8.35 mm. Hg. The distance over which this decrease takes place measured approximately 320 mm. The average decrease in pressure is 1.0 mm. Hg for every 38 mm. distance. From the foregoing data the average decrease in pressure of 1.0 mm. Hg for every 35 mm. distance is obtained.

Experiments 1, 3, 10, 13, and 17 show that the pressure in two corresponding veins is very nearly the same. In fact, the differences in the pressure of the left and right external jugular veins, on the one hand, and between the left and right femoral veins, on the other, are so slight that they might easily be due to the impossibility of adjusting the zero-point of the liquid exactly alike in both cases. Experiment 13 shows even a complete correspondence between the two femoral veins, the pressure being 5.6 mm. Hg in each vessel.

If contrasted with the arterial pressure, the pressure in the entire venous system shows only very slight variations in different animals. This statement also implies that the pressure in a certain vein does not vary greatly in different animals. The difference between the highest and lowest pressure value is greatest in the left femoral vein, but even in this instance it amounts to only 4.4 mm. Hg.

The difference in the pressure of the left external jugular vein obtained from all the experiments, amounted to 3.6 mm. Hg. It must be attributed, at least in part, to the fact that the T tube was inserted in four cases (marked *) higher in the neck. Naturally, the result was that the pressure was greater in these cases than in the others. On the whole, it may be said that a horizontal line drawn through the tip of the manubrium, marks the point central to which the pressure in this vein becomes negative and peripheral to which the pressure is positive. The "danger line" of the surgeon lies, therefore, in close proximity to the thorax.

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## Respiratory Variations in Venous Pressure.

In the preceding enumeration of the experiments only the average value of the pressure has been given, but we know that definite variations occur with every inspiratory and expiratory movement.

In Fig. 1, inserted to illustrate this statement, the respiratory variations in venous pressure were recorded by means of a Hürthle's venous manometer, connected with the external jugular vein opposite the tip of the sternum. The respiratory movements were recorded by a tambour communicating with the left pleural cavity. The downward stroke corresponds, therefore, to inspiration, the upward stroke to expiration. The time-curve, written in fifths of seconds by a Jaquet chronograph, served as the abscissa for the latter record, the zero-line of the scale as the abscissa for the former curve.

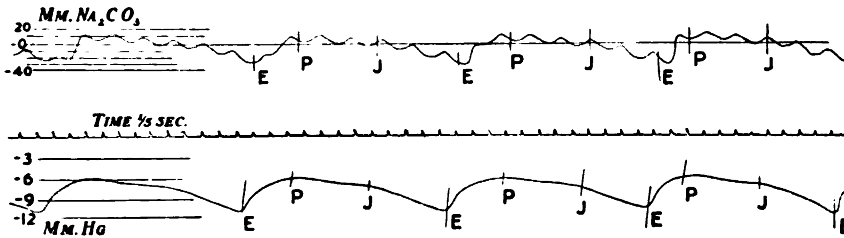


FIGURE 1. — The variations in venous pressure during normal respiration (external jugular vein).

If a comparison is made with the calibration-scale, inserted at the beginning of the venous pressure, it is seen that a considerable variation occurs. In this case the pressure varied from +12 to −23 mm. $Na_2CO_3$ solution, or 35 mm. in all (2.8 mm. Hg). It must be remembered, however, that the inspiratory movement (J to E) was in this instance deeper than usual; the greatest negative pressure at the end of inspiration amounted to 11 mm. Hg.

Normally, therefore, the lowest pressure occurs at the end of inspiration, while the highest pressure is synchronous with the end of expiration. The course of the respiratory variations in venous pressure is therefore the reverse of those occurring in the arterial system.

For the purpose of determining the amplitude of the respiratory changes in venous pressure, two manometers were employed, one of which recorded only plus pressures, while the other was arranged in

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such a way as to indicate only the values below zero. Their simultaneous action was made possible by connecting the T tube in the vein, first, with a Y tube, the branches of which led to the respective manometers.

The pressure was recorded in four different dogs in the left external jugular, facial, and femoral veins. Instead of the sodium-carbonate solution, a normal saline solution was used. The values were subsequently transferred into mm. $Na_2CO_3$ solution and mm. Hg according to the formula given previously. The respiratory movements were normal in all cases. These four experiments gave the following average results. In the left external jugular vein the pressure between its highest and lowest points showed a variation of 20 mm. $Na_2CO_3$ solution (1.6 mm. Hg). In the femoral vein the pressure between its lowest inspiratory and highest expiratory value showed a variation of 8 mm. $Na_2CO_3$ solution (0.6 mm. Hg). In the left facial vein the respiratory variations ranged from 2 to 3 mm. $Na_2CO_3$ solution (0.16 to 0.2 mm. Hg).

The greatest variation in venous pressure during inspiration and expiration is therefore shown most conspicuously in close proximity to the chest.

## Changes in Venous Pressure Resulting from Certain Experimental Procedures.

**Effect of compression of the right external jugular vein.** — In some of the experiments the right external jugular vein was tightly compressed, while the pressure was recorded in the veins of the opposite side.

The effect of this procedure always betrayed itself in a rise above normal in the left facial and left external jugular veins. The increase in the pressure was, however, more conspicuous in the peripheral vessel, the facial vein.

In Table II the normal pressure is given in relation with the pressure prevailing during the compression. It must also be mentioned that in this table, as well as in the succeeding, the experiments are numbered in accordance with those dealing with the normal pressure. The values are given in mm. Hg only.

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TABLE II.

COMPRESSION OF THE RIGHT EXTERNAL JUGULAR VEIN.

| Experiment. | Normal pressure (mm. Hg) in | | Pressure during compression (mm. Hg) in | |
|---|---|---|---|---|
| | Left facial vein. | Left external jugular vein. | Left facial vein. | Left external jugular vein. |
| 8 | 3.6 | 0.2 | 5.2 | 1.4 |
| 10 | 5.6 | 0.7 | 6.8 | 1.2 |
| 12 | 5.2 | 2.0 | 6.0 | 2.6 |
| 16 | 5.4 | 1.2 | 6.3 | 2.0 |

**Compression of both carotid arteries.** — In these experiments the common carotid arteries which had previously been placed in ligatures, were raised and compressed between the fingers.

The effect on the pressure in the left facial and jugular veins was generally not immediate. Three or four inspirations were required before the maximal decrease in pressure appeared.

Table III is arranged in the same manner as the preceding; the normal pressure is inserted together with the pressure prevailing at the end of the compression.

TABLE III.

COMPRESSION OF BOTH CAROTID ARTERIES.

| Experiment. | Normal pressure (mm. Hg) in | | Pressure at end of compression (mm. Hg) in | |
|---|---|---|---|---|
| | Left facial vein. | Left external jugular vein. | Left facial vein. | Left external jugular vein. |
| 8 | 3.6 | 0.2 | 2.2 | −0.3 |
| 9 | 4.0 | −0.8 | 2.4 | −2.0 |
| 10 | 5.6 | 0.7 | 4.0 | 0.08 |
| 12 | 5.2 | 2.0 | 3.0 | 1.0 |
| 16 | 5.4 | 1.2 | 4.0 | 0.16 |

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**Compression of the femoral artery.** — The femoral artery was compressed with the forceps opposite the T tube in the vein, *i. e.* about three to four cm. below the groin.

Although a gradual fall in pressure always resulted, the decrease was surprisingly slight. Under these conditions the pressure apparently assumes the value of the pressure prevailing in the next, more central venous trunk, the iliac vein.

TABLE IV.

COMPRESSION OF THE FEMORAL ARTERY.

| Experiment. | Normal pressure (mm. Hg) in femoral vein. | Pressure during compression (mm. Hg) in femoral vein. |
|---|---|---|
| 9 | 5.6 | 4.9 |
| 11 | 3.2 | 2.4 |
| 14 | 6.3 | 5 6 |

**Effect of stimulation of the vagus.** — The right vagus nerve was stimulated in a number of instances with a tetanic current of sufficient strength to cause a gradual cessation of the action of the heart.

The effect of this procedure always betrayed itself in a rise in pressure in all the veins. Attention has been called to this fact previously by Klemensiewicz[1] who observed the changes in pressure in the femoral artery and vein during the stimulation of this nerve.

The rapidity of the rise is proportionate to the strength of the stimulus. If the heart is stopped slowly, the pressure increases at an equally slow rate. If, however, the current is very strong, so that an almost instantaneous stoppage results, the pressure rises very quickly at first.

Naturally, the increase in pressure is also determined in a large measure by the respiratory movements, but of course only in case they do not cease in consequence of the stimulation. Supposing that a rise of 30 mm. soda solution has taken place in the external jugular veins solely on account of the backward stagnation of the column of

[1] KLEMENSIEWICZ, R.: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften. Mathematisch-naturwissenschaftliche Classe. Wien, 1886, Sect. 3, pp. 66–84.

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blood from the right auricle, the next inspiration will cause a decided fall in the level of the liquid, proportionate to the depth of this movement. But, as soon as the expiratory phase sets in, the pressure rises again, until its value is much greater than formerly. This phenomenon will be repeated with every respiratory movement occurring during the passive state of the heart.

It must be explained in the following manner: The inspiratory movement draws a considerable quantity of blood into the central venous trunks, but, as the blood is not propelled onward, this amount is simply added to that already accumulated here. The pressure rises, therefore, in a corresponding measure, not steadily, however, but by degrees.

The heart was kept in the passive state for a comparatively long time. In Experiments 8 and 10 the stimulation caused an almost immediate cessation of respiration, with the chest in the expiratory position. A weaker current being used in Experiments 9, 11, and 12, at least one complete respiratory phase occurred after the heart had ceased to beat. In Experiment 11, four respiratory movements took place during the passive state of the heart. The results clearly show

TABLE V.

STIMULATION OF THE VAGUS.

| Experiment. | Pressure in | Normal pressure, mm. Hg. | Pressure at end of stimulation, mm. Hg. |
|---|---|---|---|
| 8 | Left external jugular vein | 0.2 | 3.6 |
| | Superior v. cava (dist. p.) | −2.0 | 1.6 |
| | Left femoral vein | 4.4 | 7.3 |
| 9 | Left facial vein | 4.0 | 8.0 |
| | Left external jugular vein | −0.8 | 4.8 |
| | Superior v. cava (dist. p.) | −2.2 | 3.2 |
| | Left femoral vein | 5.6 | 8.4 |
| 10 | Left facial vein | 5.6 | 7.2 |
| | Left external jugular vein | 0.7 | 4.4 |
| | Superior v. cava (dist. p.) | −0.16 | 2.8 |
| | Left femoral vein | 4.4 | 6.8 |
| 11 | Left external jugular vein | −0.4 | 5.6 |
| | Superior v. cava (dist. p.) | −1.7 | 3.2 |
| | Left femoral vein | 3.2 | 6.4 |
| 12[1] | Left facial vein | 5.2 | 9.6 |
| | Left external jugular vein | 2.0 | 5.6 |
| | Superior v. cava (dist. p.) | −1.4 | 4.8 |
| | Left femoral vein | 5.0 | 8.0 |

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that the increase in pressure is particularly noticeable in those cases in which the respiratory movements were not abated completely.

After the stimulation the pressure decreased gradually to normal, every heart-beat causing a distinct fall in the pressure.

In the experiments contained in Table V, the normal pressure is given in relation with the pressure prevailing at the end of the period of stimulation.

**Changes following section of the vagi.** — This procedure was invariably followed by a fall in venous pressure, but the decrease was not equally evident in all cases. Experiments 14, 15, and 16 (Table VI) show a very decided effect; in Experiments 13 and 18, on the other hand, the decrease is less conspicuous.

TABLE VI.

THE CHANGES FOLLOWING SECTION OF THE VAGI.

| Experiment. | Pressure in | Normal pressure. mm. Hg. | Pressure after nerve section. mm. Hg. | Pressure in left pleural cavity. mm. Hg. |
|---|---|---|---|---|
| 13 | Left external jugular vein | 0.2 | −0.4 | −3 to −18.5 |
| | Left femoral vein | 5.6 | 3.2 | Resp. slow. |
| 14 | Left external jugular vein | −0.8 | −2.0 | Resp. frequent. |
| | Superior v. cava (dist. p.) | −2.4 | −3.2 | |
| | Left femoral vein | 6.3 | 3.6 | |
| 15 | Left external jugular vein | 2.4 | −2.0 | −6.5 to −21 |
| | Superior v. cava (dist. p.) | −0.4 | −4.4 | Resp. frequent, |
| | Left femoral vein | 6.2 | 2.4 | deep. |
| | Left saphenous vein | 6.8 | 2.8 | |
| 16 | Left external jugular vein | 1.2 | −0.8 | −17.5 |
| | Superior v. cava (dist. p.) | 0.0 | −4.0 | Resp. frequent. |
| | Left femoral vein | 5.7 | 3.2 | |
| 18 | Left external jugular vein | −0.5 | −0.4 | Resp. slow, deep. |
| | Superior v. cava (dist. p.) | −1.7 | −1.6 | |
| | Left femoral vein | 6.4 | 6.0 | |

This slight inconsistency in the results cannot be ascribed to a variable increase in the frequency of the heart, because, even if a larger quantity of blood leaves the heart, an equal amount must be returned to this organ. Evidently it must be attributed wholly to differences in the rate and depth of the respiratory movements.

The deep inspiratory movements usually following section of these nerves, naturally are accompanied by a very decided fall in venous pressure. When the expiratory phase sets in, followed by the long

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pause, the pressure increases again and rises the higher, the longer the interval between the successive respirations. In brief, the respiratory variations in pressure are extremely conspicuous.

It may readily be assumed that the pressure must be lower in those cases in which the respiratory movements are more frequent and deep. The longer the duration of the respiratory pause, the higher will the pressure become during this interval. These differences in the frequency and depth of respiration are always present after section of the vagi, and undoubtedly are responsible for the variable conspicuousness of the decrease in pressure observed in Table VI.

The excursions of the liquid being slow, so that secondary oscillations could not occur, the highest and lowest pressure-values were recorded, the average being obtained subsequently by calculation. The pressures were read for about two minutes after the section of these nerves.

**Changes resulting from opening the chest (suspended respiration).** — The normal venous pressure having been obtained, the chest was opened, and the pressure again recorded about one minute after this operative procedure. In Experiments 9 and 12 the tissues of two or three intercostal spaces were cut and torn sufficiently, so that the "reflex" respiratory movements, following the collapse of the lungs, could no longer influence the level of the liquid in the manometers. In the other experiments, the abdominal cavity was opened first by an incision in the linea alba. The diaphragm was divided subsequently along its anterior edge.

The heart-beats were counted before and after this operative procedure. They were found to possess normal force and frequency at least for some time after the collapse of the lungs. The very decided changes in pressure noticed after the suspense of respiration, therefore cannot be attributed to differences in the action of the heart.

The pressure in all the veins rose immediately with the first rush of air into the chest. The increase continued gradually for about thirty or forty seconds, when the maximal height was attained. The rise, as is evident in Table VII, was often very considerable. All the negative pressures disappeared, at least as far centrally as the distal portion of the superior vena cava. The important question, whether the negative pressure is also destroyed by this procedure directly in and near the entrance to the right auricle, could not be

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determined by the method employed in this investigation, because the correct adjustment of the manometer with the chest closed is impossible. An approximate adjustment is not permissible in this case, because under these conditions even the most minute changes must be taken into account.

These experiments clearly show that the negative pressure normally prevailing in the central venous trunks is wholly dependent on the rate and depth of the respiratory movements (aspiration of the chest). If the right auricle and ventricle are able to produce an active negative pressure independently, this pressure must be confined to the limits of the heart. As is shown in Table VII, the pressure in the distal portion of the superior vena cava on opening the chest rose as an average to +19.5 mm. soda solution, or 1.55 mm. Hg. Considering the distance between this point and the right auricle, and the fall in pressure per millimetre distance (see page 202), we must conclude that the pressure was positive even at the entrance to the right side of the heart.

On opening the abdomen no decided change in pressure could be observed. An increase, amounting to 5 mm. soda solution, was clearly recognized in only one instance (Experiment 10). It took place in the distal end of the superior vena cava.

Table VII contains the normal pressure in relation with the pressure prevailing shortly after the chest had been opened and the respiration suspended.

TABLE VII.

THE CHANGES RESULTING FROM OPENING THE CHEST (SUSPENDED RESPIRATION).

| Experiment. | Pressure in | Normal pressure, mm. Hg. | Pressure after opening chest, mm. Hg. |
|---|---|---|---|
| 9 | Left facial vein | 4.0 | 6.4 |
| | Left external jugular vein | −0.8 | 4.8 |
| | Superior v. cava (dist. p.) | −2.2 | 2.4 |
| | Left femoral vein | 5.6 | 7.2 |
| 10 | Left external jugular vein | 0.7 | 2.9 |
| | Superior v. cava (dist. p.) | −0.16 | 1.6 |
| 11 | Left external jugular vein | −0.4 | 3.3 |
| | Superior v. cava (dist. p.) | −1.7 | 1.4 |
| | Left femoral vein | 3.2 | 5.2 |
| 12 | Left facial vein | 5.2 | 6.5 |
| | Left external jugular vein | 2.0 | 3.6 |
| | Superior v. cava (dist. p.) | −1.4 | 0.8 |
| | Left femoral vein | 5.0 | 6.0 |

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If after this period of suspended respiration, the lungs of the animal were inflated artificially, another conspicuous change took place. Normally the pressure falls during inspiration and rises during expiration (see Fig. 1). If artificial respiration is employed the reverse phenomenon occurs, *i. e.* a rise on inspiration and a fall during expiration.

The upper line of Fig. 2 represents the expansion of the lungs by the artificial air-current. The inspiratory phase continues from J to E and the expiratory period from E to J. The ordinates are indicated by the same letters in the curve of the venous pressure. The respiratory phases were recorded by a tambour, connected by means of a T tube with the tracheal cannula. In the case now under consideration

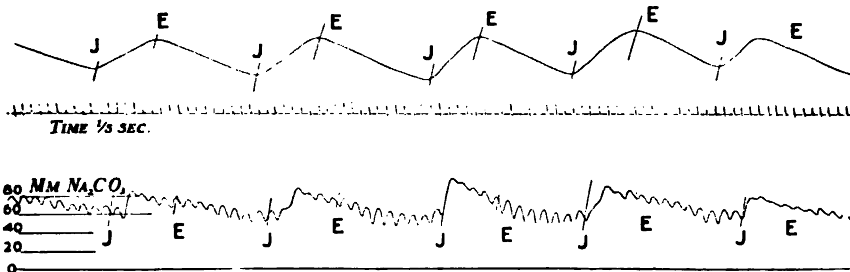


FIGURE 2. — The variations in venous pressure during artificial respiration (external jugular vein).

the stop-cock, interposed to lessen the excursions of the writing lever of the tambour, was closed somewhat too tightly. The inspiratory phase is therefore prolonged beyond its real duration. The venous pressure was recorded as in Fig. 1, by a Hürthle's venous manometer, connected with the external jugular vein in close proximity to the chest.

As Figs. 1 and 2 were obtained from the same animal, it might be well to compare the pressure-values of these two curves. In Fig. 1, the pressure varied from −23 to +12 mm. $Na_2CO_3$ solution. The chest being opened, the pressure rose considerably above normal, namely, to 63 mm. $Na_2CO_3$ solution (5.0 mm. Hg). Artificial respiration being employed subsequently, the pressure varied from 58 to 83 mm. $Na_2CO_3$ solution.

Thus, the lowest pressure value is reached in the latter case not at the end, but at the beginning of inspiration. In a similar manner, the highest pressure which normally occurs at the end of expiration, is in this case synchronous with the end of inspiration.

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The excursions of the liquid in the manometers are proportional to the frequency and the force of the artificial currents of air. If a great amount of air is forced into the lungs, a corresponding increase in venous pressure follows. The effect of artificial respiration seems to be purely mechanical. During the inspiratory phase, the pulmonary vessels are compressed, while during expiration they are more free, allowing during the latter period a greater flow from the right side of the heart.

## Summary.

Among the conclusions derived from the preceding tables, the following should be noted particularly:

1. The average venous pressure in eighteen dogs, varying in weight from 6 to 24.5 kilos, was as follows:

| | | |
|---|---|---|
| Left facial vein | 5.12 | mm. Hg |
| Left external jugular vein | 0.52 | " |
| Right external jugular vein | −0.08 | " |
| Sup. vena cava, distal portion | −1.38 | " |
| Sup. vena cava, near entrance to right auricle | −2.96 | " |
| Right brachial vein | 3.90 | " |
| Left femoral vein | 5.39 | " |
| Right femoral vein | 5.42 | " |
| Left saphenous vein | 7.42 | " |

2. The pressure decreases gradually from the periphery toward the centre of the circulatory system. The fall in pressure amounts to 1.0 mm. Hg every 35 mm. distance.

3. The pressure in two corresponding veins is very nearly the same.

4. The pressure in a certain vein does not vary greatly in different animals. As compared with the arterial system, the variations in pressure, in the entire venous system, are very slight.

5. In an animal with normal heart action and respiration, negative pressure appears first in very close proximity to the thoracic cavity.

6. Compression of the right jugular vein causes a decided rise in the pressure of the opposite facial and jugular veins. Compression of both carotid arteries is followed by a distinct decrease in the pressure of the veins just mentioned. Compression of the femoral artery causes a slight fall in the pressure of the corresponding vein.

7. Stoppage of the heart by stimulation of the vagus is followed by a very decided increase in venous pressure, due to the accumulation

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of the blood in front of the right side of the heart. The rise is more conspicuous in the central venous trunks.

8. Section of both vagi is followed by a fall in venous pressure, which, if the respiratory movements are favorable, may become extremely pronounced.

9. After opening the chest, the pressure rises far above normal in all the veins. All negative pressures disappear, at least as far centrally as the entrance to the right auricle.

10. Normally the pressure falls during inspiration and rises during expiration. Artificial respiration being employed, the reverse phenomenon results.

The respiratory variations in venous pressure are most conspicuous in close proximity to the chest.

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# A STUDY OF THE PHYSIOLOGICAL ACTION AND TOXICOLOGY OF CÆSIUM CHLORIDE.

By G. A. HANFORD.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

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ALTHOUGH cæsium belongs to a group of elements of which several members play an important rôle among the inorganic compounds that occur in living tissues, its physiological action has received little attention. There are a few researches on record in which the comparative action of the various salts of the alkali metals have been studied. Thus Richet[1] determined the absolute and relative toxicity of the latter on the frog's heart, and expressed their relations in the following table:

| | Molecular weight. | Absolute toxicity. | Molecular toxicity. |
|---|---|---|---|
| Cæsium | 133.0 | 100 | 0.74 |
| Rubidium | 85.4 | 43 | 0.51 |
| Lithium | 7.03 | 27 | 3.9 |
| Potassium | 39.15 | 26 | 0.67 |
| Ammonium | 17.04 | 25 | 1.4 |

[1] RICHET: Dictionnaire de physiologie, 1898, iii, p. 73, where the earlier literature is cited. The figures express the quantity of metal per litre of which four drops suffice to paralyze the frog's heart.



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From these figures it appears that equimolecular solutions of cæsium possess a toxicity comparable with that of potassium and rubidium, the other alkali compounds standing in a group by themselves.

Brunton and Cash[1] made a comparative study of the action of the alkali chlorides upon muscles and nerves. According to them, neither rubidium nor cæsium paralyzes the motor nerves until large doses are employed. The contractile power and also the duration of contraction is increased by cæsium. The numerous other details ascertained need not be repeated here.

Ringer[2] has contrasted the action of cæsium and rubidium salts with that of potassium salts by perfusion of the ventricle of the frog's heart. He concluded that rubidium, which is chemically closely allied to cæsium, is almost identical in its physiological effects with potassium, from which cæsium differs markedly. The chloride of cæsium showed a behavior more like the salts of barium and strontium. In criticism of these results, Blake[3] states that rubidium and cæsium agree with the other metals of the group in their action, potassium being the single exception. He observed that "when injected into the arteries, the cæsium salts cause a sort of cataleptic state of the voluntary muscles, and a curious slowing of the reflexes, two or three seconds, for instance, elapsing between irritating the cornea and closure of the lids." He adds that "this has not been observed in any other of the monovalent metals."

In an effort to establish experimentally a relationship between the chemical properties and the physiological reactions of the alkali metals, Botkin[4] studied the effects induced on blood-pressure by the different alkali chlorides. In the case of rubidium chloride, intravenous injection of 0.03 gram per kilo of body weight in the dog was followed by a marked rise in arterial pressure frequently amounting to one hundred mm. Hg, whether the animal was curarized or not. The high pressure was maintained from one-half to four minutes, according to the dose. The rate of the heart beat was not always affected in the same way. The force of the cardiac contractions, however, was increased and remained so, even after the pressure fell again. Frequently the rise in pressure was preceded by a slight initial fall.

[1] Brunton and Cash: Philosophical transactions of the Royal Society of London, 1885, clxxv, p. 197.

[2] Ringer: Journal of physiology, 1883, iv, p. 370.

[3] Blake: Journal of physiology, 1884, v, p. 124.

[4] Botkin: Centralblatt für die medicinische Wissenschaften, 1885, xxiii, p. 849.

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The injection of 0.04 gram per kilo into a curarized animal quickly caused the heart to stop in diastole. These phenomena were likewise obtained after section of the spinal cord, although a larger dose was necessary. The rise in pressure was not prevented by section of the vagi or by atropine.

With cæsium chloride analogous results were obtained, although almost double the dose noted for the rubidium salts was necessary. In slowing the heart, cæsium chloride was apparently more effective than the rubidium compound. Botkin also observed that rubidium and cæsium resembled potassium in their action on the excised frog's heart, cæsium chloride being the least toxic.

Harnack and Dietrich[1] have also compared the action of rubidium and cæsium chloride. They report that these salts have similar actions on the striated muscle of the frog, differences in intensity alone being noted. Thus because cæsium chloride diffuses less rapidly, its physiological action is less marked. The essential feature is a paralysis preceded by an initial increase in irritability and contractile power. The results differ from those reported by Brunton and Cash,[2] who observed no initial excitability, but only an increasing paralysis.

## General Effects of Cæsium Chloride.

In the present investigation, the physiological action of cæsium chloride has been studied on the frog, rabbit, cat, dog, and man. A few observations have been made on the effects of this salt upon isolated tissues and cells from the frog in comparison with the reaction of equimolecular solutions of sodium chloride. In experiments with the mammals, attention was directed to the toxicological effects of cæsium chloride, its action on the circulation, the mode of elimination, and the effects on metabolism. The cæsium chloride used in all the experiments was obtained through the kindness of Prof. H. L. Wells of the Sheffield Chemical Laboratory. It was prepared as follows: Pollucite from Paris, Maine, was extracted with hydrochloric acid, and precipitated with antimony chloride as a double salt, $3CsCl_2SbCl_3$. This compound was then decomposed with ammonia, and the resultant cæsium chloride treated with nitric acid to form cæsium nitrate.

[1] Harnack and Dietrich: Archiv für experimentelle Pathologie und Pharmakologie, 1885, xix, p. 153.

[2] Brunton and Cash: Philosophical transactions of the Royal Society of London, 1885, clxxv, p. 197.

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With iodine and hydrochloric acid, this in turn gave a double salt of cæsium chloride and iodine ($CsCl_2I$), which was recrystallized, and upon ignition yielded pure cæsium chloride. Spectroscopic examination indicates that the salt obtained is unusually pure.

**Experiments on the frog.** — Frog's blood taken directly from the heart was dropped into $\frac{n}{8}$ solutions of pure cæsium chloride and sodium chloride respectively. Microscopic examinations made at intervals showed no marked difference in the behavior of the cells in the two solutions. The same is true of ciliated epithelial cells taken from the roof of the mouth. In some experiments the movements of the cilia seemed to diminish in rapidity somewhat earlier in the cæsium chloride solution. Carefully isolated gastrocnemius or sartorius muscles were immersed in freshly prepared $\frac{n}{8}$ solutions of the two salts. The spontaneous contractions described by Loeb[1] were quickly manifested. They always continued for a longer time in the muscle immersed in sodium chloride. After the spontaneous contractions had ceased, irritability to electrical stimulation disappeared first in the muscle exposed to the cæsium solution.

When two leg-nerve preparations were made from the same animal, and the attached sciatic nerves were exposed to one of the solutions, the loss of irritability to electrical stimulation was evident in the nerve dipped in $\frac{n}{8}$ cæsium chloride solution sooner than in the one immersed in the sodium chloride solution. For example, in one experiment the cæsium chloride nerve failed to respond at the end of three hours, while the nerve in sodium chloride still reacted faintly after eight hours. When neither nerve was longer excitable, the gastrocnemii, which reacted to direct stimulation, were in turn immersed in the corresponding solutions. Here again response to stimulation failed first in the muscle exposed to the cæsium chloride.

When cæsium chloride is introduced subcutaneously in the frog, a complete paralysis results. The dose necessary to produce this effect was found to vary from one to two-and-a-half milligrams per gram of body weight. The paralysis ensued gradually, and in no instance was any preliminary excitation noted except such as might be referred to a local irritation at the seat of injection. The paralysis usually first manifested itself in the fore legs, which sometimes became stiff and sometimes remained limp. The reflexes were usually better in the hind legs than in the fore legs. Electrical stimulation of the exposed

[1] Loeb: Beiträge zur Physiologie; Festschrift für A. Fick, 1899, p. 101; also This journal, 1900, iii, p. 136.

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sciatic nerve of a paralyzed frog failed to occasion a muscular response, although, in the cases examined, the muscles were still irritable directly. The symptoms elicited in the doses mentioned resemble more closely those described by Brunton and Cash than those noted by Harnack and Dietrich. The initial excitability described by the latter was not noted in the present experiments.

The reflexes were studied by Türck's method. In the pithed normal frog, a response was obtained by immersion in 0.4 per cent $H_2SO_4$ for from four to eight seconds. Half an hour after the onset of cæsium paralysis, responses were delayed half a minute, this latent period increasing as the period of intoxication continued. In the few experiments undertaken to locate the specific action of the salt, the results seemed to indicate a reaction on both muscle and nerves in accordance with the observations of Brunton and Cash.

**Experiments on rabbits.** — In this animal doses of cæsium chloride as large as one and a half grams per kilo of body weight, introduced subcutaneously, failed to provoke any noticeable symptoms. This quantity, as will be seen later, is considerably greater than the fatal dose for the cat or dog. Within two hours, the urine removed by catheterization showed the characteristic cæsium spectrum; and in one animal, with the dose mentioned, the spectroscopic test for cæsium could still be obtained in the urine after nine days. The fæces also gave the test.

A rabbit which received two hypodermic doses of one gram per kilo of body weight died seven hours after the first injection. Soon after the second dose was administered, the animal began to lose control of its hind legs, and severe diarrhœa ensued. An increasing weakness, leading up to complete paralysis, was followed by death. The post-mortem appearance of the organs was not unusual. Cæsium was detected in the stomach contents, various portions of the intestinal contents, and the urine.[1]

**Experiments on cats.** *Feeding trial.* — A medium-sized cat was given two hundred milligrams of cæsium chloride in one hundred cubic centimetres of milk each morning. This was continued for eight days, when the animal was killed. The food was taken readily and during the experiment no toxic or unusual symptoms were noted, except a tendency toward diarrhœa. Up to the time of

[1] The stomach contents were carbonized and extracted with water. The extract was concentrated and then tested spectroscopically. The other materials were tested directly.

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the animal's death the urine each day showed a progressively stronger cæsium reaction with the spectroscope. The fæces also gave the characteristic spectrum. The post-mortem appearance of the organs was normal in every respect; they gave pronounced cæsium reactions when tested spectroscopically, showing that cæsium chloride readily passed into the various organs and tissues of the body.

*Subcutaneous injection.* — The effect of subcutaneous injection was next studied. In one animal, weighing two and one-half kilos, a dose of one and one-quarter grams (one-half gram per kilo body weight) proved fatal in seven days. Within a few minutes after the injection, the animal became restless, and soon intensely excited, biting at anything within reach. This lasted about two hours, until fatigue supervened. These peculiar symptoms are probably referable to local irritation at the point of injection, rather than to any specific action of the cæsium salt. Both the urine and the fæces gave good cæsium reactions up to the time when the animal died. For four days after the injection, the cat improved, finally taking food previously refused, so that its actions appeared quite normal. However, from this time on, an increasing weakness was noted, showing itself in a disinclination to stand, and a tendency to favor the hind legs. During the last two days, the animal refused food, and seemed unable to control its legs, staggering when attempting to move. This lack of control gradually extended towards the head, until shortly before death the animal was scarcely able to move any part. Its reflexes showed a corresponding progressive disappearance. Thus the paralysis evoked seemed to begin in the extremities, especially the hind legs, and gradually to spread forward until it involved the entire body. As will be seen later, similar progressive paralysis was observed in the dog. Death ensued when the paralysis was complete, all muscles being relaxed. On post-mortem examination, there was found considerable œdema of the subcutaneous connective tissue. The internal organs were carbonized, extracted with water, and the extract evaporated for spectroscopic examination. The liver and intestine gave the strongest reaction; the kidney, spleen, and pancreas gave fair reactions; the brain and lung showed the spectrum faintly. The intestinal contents also gave a a good reaction; it is thus evident that cæsium may be excreted through the gut.

In two other animals receiving the same dose, similar symptoms were provoked, with the exception that recovery ultimately followed.

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The first one of these (three and seven-tenths kilos in weight) having received 1.85 grams, was killed at the end of a month. No cæsium reaction could be obtained from any part of the animal. The second cat (three and four-tenths kilos in weight) received 1.7 grams; in this animal the characteristic symptoms described were less pronounced. The urine and fæces ceased to show the cæsium spectrum after ten days. Further details regarding the toxic symptoms produced by cæsium chloride are given in the descriptions of the metabolism experiments.

*Effects upon the circulation.* — In a cat of two and eight-tenths kilos body weight, arterial pressure was measured with a mercurial manometer. Anæsthesia was maintained by administration of A. C. E. mixture, after a subcutaneous injection of forty milligrams of chloralose. A total dose of two and one-tenth grams (three-quarters of a gram per kilo body weight) of cæsium chloride was injected into the jugular vein during the experiment. After receiving about two-thirds of this amount, the animal breathed more deeply and slowly. In a few minutes both the pulse and breathing became very irregular. With this irregularity in the pulse came also a slight fall in pressure. Within seven minutes the latter had given place to a marked rise. At the same time the heart beat increased in force, becoming irregular. After the pressure returned to the normal, the remaining one-third of the cæsium solution was introduced, resulting in a second marked rise in pressure, and finally death due to cardiac failure. This took place before the injection was finished. The urine (from the bladder), the bile, the thyroid gland, and intestinal contents from different portions, all gave good cæsium reactions spectroscopically. The stomach contents gave the reaction after carbonization.

**Experiments on dogs.** *Subcutaneous injections.* — A young bitch weighing eleven and three-tenths kilos received eight and one-half grams of cæsium chloride (three-quarters of a gram per kilo body weight). The initial symptoms of local irritation were observed, though they were not so marked as in the case of the cats described previously. Food was refused, and within two days the animal became indifferent to its surroundings, remaining in any position in which it was placed. Thus, when placed standing in a corner, it would remain so until fatigue intervened. On the fourth day the animal appeared very weak, and was unable to control its legs. Whereas heretofore the symptoms seemed to indicate indifference rather than fatigue, now the animal appeared weak and exhausted, and when

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placed upon a table was unable to hold up its head. Diarrhœa and bloody urine were symptoms of the last few days previous to death. The animal died in complete paralysis, all muscles being relaxed. Upon post-mortem examination the subcutaneous tissue was found highly congested, as was also the spleen. The blood clotted normally. All the organs tested, except the brain, gave a pronounced cæsium reaction.[1]

In another experiment a dog weighing ten kilos received five grams (one-half gram per kilo body weight) of cæsium chloride; and after three days, a second dose of seven and one-half grams (three-quarters of a gram per kilo body weight) was injected. The first dose provoked the usual initial symptoms of local irritation and uneasiness, loss of appetite, and diarrhœa. Beyond this nothing unusual was noted. After the second dose, however, the animal was more distressed, and refused food for two days, being quite sick and subject to frequent defæcation and vomiting. This animal recovered somewhat after a few days, and would eat meat. The urine and fæces showed bright cæsium bands when tested spectroscopically. At the end of ten days, the animal was killed. During the two or three days previous, signs of weakness and loss of control already noted in the other animals were evident. There was no œdema of the tissues, and the internal organs presented no unusual appearance. The cæsium spectrum was plainly shown by all the organs and important tissues, and by both the urine and fæces.

From these injection experiments upon both the dog and cat, it seems demonstrated that cæsium chloride enters all the tissues and organs, and is excreted by way of the intestine as well as by the kidneys. Although the foreign element soon appears in the urine and fæces, complete elimination follows only slowly, the last traces not disappearing until several days have elapsed. A more permanent retention in specific tissues such as occurs with iodine in the thyroid gland could not be detected. A marked disturbance of the gastro-intestinal tract was noted in all animals to which larger doses were administered by any channel.

*Effect on the circulation.*—To study the effect of the salt upon the circulation in the dog, two experiments were made. In the first, a bitch of seven kilos weight received three small doses and a large one. Anæsthesia was maintained by the injection of forty milligrams of

[1] These organs were: kidney, liver, spleen, lung, intestine (with contents), muscle, and brain.

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morphine sulphate and four milligrams of atropine, followed by A. C. E. administration. Blood-pressure was measured by a mercurial manometer connected with the carotid. The lymph flow was measured by means of a cannula introduced into the thoracic duct. The urine was obtained by catheterization. The injection of one-tenth gram into the femoral vein caused no noticeable change of any sort. After a few minutes, one-half gram more was injected, which caused a slightly more rapid flow of lymph. The giving of a third dose of three-quarters of a gram had no further effect. Finally two and one-half grams were injected, which caused the animal's death immediately from cardiac failure. A few respiratory efforts were noted after the heart had stopped beating.

The urine taken after the second injection (one-half gram) showed a faint cæsium spectrum. From this time on the reaction grew stronger. After the second injection, the lymph also gave the spectrum test, which grew stronger as more was introduced. The liver, kidney, and thyroid gland all gave good cæsium reactions.

In the second experiment a dog weighing fourteen kilos was used. Anæsthesia was maintained by the injection of eighty milligrams of morphine sulphate and eight milligrams of atropine, followed by the administration of A. C. E. mixture. Cannulæ were inserted into the lymphatic duct, both Whartonian ducts, both ureters, the right femoral artery, and the right jugular vein. The chorda-lingual nerves were both cut. The lymph flow was normal, as was also the urinary secretion. A dose of fifty cubic centimetres of a four per cent solution (two grams) was injected, which brought out the usual symptoms, though less marked than in the previous experiment. There was an initial slight fall followed by a rise in pressure, great irregularity in the heart beat, and deep, labored breathing. After the effect of this dose had disappeared, a second one of twenty-eight cubic centimetres of the same solution (one and twelve-hundredths grams) was injected, causing similar effects. Pilocarpine hydrochloride was then introduced into the circulation to provoke salivary flow. The second drop of saliva from each submaxillary gland showed the spectrum of cæsium, as did the saliva dripping from the mouth and the secretion from the nose. Within twenty minutes from the first injection cæsium could be detected in the lymph flowing from the thoracic duct. As the experiment progressed, the rate of lymph-flow increased about fifty per cent. Within about fifteen minutes after injection, the salt could be demonstrated in the urine flowing from either ureter.

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The appearance of this salt in the lymph was more tardy than in the case of some salts, as reported by other investigators. Thus, Tschirwinsky [1] observed sodium salicylate in the lymph within four to seven minutes; Cohnstein [2] found four to five minutes to intervene before potassium or sodium ferrocyanide reappeared; and Mendel [3] noted about the same length of time for sodium iodide.

## Metabolism Experiments.

*Methods.* — The experiments were performed on healthy full-grown dogs ranging in weight from five to fifteen kilos. The animals were kept in cages suitable for the separate collection of solid and liquid excreta. When possible, the animals were catheterized every day, and always on the final day of a period. The urine for twenty-four hours was combined, and its volume, etc., were noted. The fæces, when dried and weighed, were analyzed for nitrogen and fat (ether-extract). The dogs were fed upon a mixed diet of fresh lean beef, cracker dust, lard, and water. The meat was prepared as described by Chittenden and Gies,[4] and usually contained about 3.5 per cent N. The cracker dust and lard were commercial brands, the former usually containing 1.6 per cent N, the latter 0.07 per cent N. The nitrogen content of all the food and the excreta was carefully determined by the Kjeldahl-Gunning process. In the urine total $SO_3$ was determined gravimetrically by periods in composite samples; $P_2O_5$ and Cl were estimated for each day, the former by titration with uranium nitrate, and the latter by the Volhard-Salkowski method.

*First experiment.* — The animal used was a dog of twelve kilos. It failed to attain perfect nitrogenous equilibrium; but the experiment may serve as a preliminary illustration. During the experiment, which consisted of three periods of eight days each, the dog was fed daily:

250 grams meat,
40 " lard,
50 " cracker dust,
300 " water.

During the second period one-half gram of cæsium chloride (about forty-two milligrams per kilo body weight) was added to the daily

[1] Tschirwinsky: Centralblatt für Physiologie, 1895, ix, p. 49.
[2] Cohnstein: Archiv für die gesammte Physiologie, 1895, lix, p. 509.
[3] Mendel: Journal of physiology, 1896, xix, p. 227.
[4] Chittenden and Gies: This journal, 1898, i, p. 5.

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diet. There was a deficiency in the nitrogen output as compared with the intake. The weight of the animal remained the same practically throughout the experiment, and no unusual symptoms were noted. There was also no material difference in the amounts of $SO_3$, $P_2O_5$, and Cl excreted during the three periods. The fat content of the fæces was not altered. A summary of the results obtained is given below.

EXPERIMENT I. SUMMARY, AVERAGE PER DAY.

| Periods, 8 days. | Total Nitrogen. | | | Urine. | | | | | Fæces. |
|---|---|---|---|---|---|---|---|---|---|
| | In-gested. | Ex-creted. | Balance. | Vol. | Nitro-gen. | $SO_3$. | $P_2O_5$. | Cl. | Nitro-gen. |
| | grams. | grams. | grams. | c.c. | grams. | grams. | grams. | grams. | gram. |
| Fore | 9.689 | 9.475 | +0.214 | 404 | 9.024 | 1.783 | 1.143 | 1.427 | 0.451 |
| Cæsium | 9.670 | 8.870 | +0.800 | 380 | 8.576 | 1.982 | 1.333 | 1.459 | 0.294 |
| After | 9.650 | 8.295 | +1.355 | 378 | 8.001 | 1.815 | 1.302 | 1.302 | 0.294 |

*Second experiment.* — After two weeks, the dog of the first experiment was used again, receiving the same diet as in the former trial. A fore period, lasting seven days, preceded one of five days, in which three grams of cæsium chloride (two hundred and fifty milligrams per kilo body weight) were given daily. At the end of this time the animal appeared languid, but otherwise no unusual symptoms were noted. Then five grams were given, which caused the animal to vomit and defæcate profusely. The dog appeared to suffer from intense gastro-intestinal irritation, scarcely rising from the floor of the cage. Blood was found in small amounts in the fæcal matter. From this time the animal received no more cæsium, and was allowed to recover. For two days, food was rejected and the same symptoms of weakness, dulness, and unsteadiness in the legs, which were noted in the injection experiments, were shown. The animal now began to take food, and gradually returned to a normal condition. No greater difference in the amounts of nitrogen, $SO_3$, $P_2O_5$, and Cl excreted were noted than in the first experiment. The data are given in the following tables :

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EXPERIMENT II. FORE PERIOD.

| Day of exp. | Body wt. | Food. | Urine. | | | | | | | Fæces. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | Vol. | Spec. grav. | Re-action to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. |
| | kilos. | grams. | c.c. | | | grams. | grams. | grams. | grams. | grams. | grams. |
| 1 | 11.9 | 9.87 | 505 | 1.022 | acid | 9.404 | | 1.243 | 1.613 | | |
| 2 | 11.8 | 9.87 | 445 | 1.026 | " | 10.118 | | 1.638 | 1.391 | | |
| 3 | 11.8 | 9.87 | 365 | 1.030 | " | 8.851 | | 2.203 | 1.408 | | |
| 4 | 11.8 | 9.87 | 428 | 1.024 | " | 9.243 | 15.561 | 1.356 | 1.333 | 99 | 5.049 |
| 5 | 11.8 | 9.87 | 455 | 1.020 | " | 8.182 | | 1.435 | 1.448 | | |
| 6 | 11.8 | 9.87 | 405 | 1.023 | " | 8.597 | | 1.356 | 1.316 | | |
| 7 | 11.8 | 9.87 | 270 | 1.027 | " | 6.753 | | 1.299 | 1.189 | | |
| | | | | N of urine . | | 61.148 | | | | | |
| | | | | N of fæces . | | 5.049 | | | | | |
| Totals . . . | | 69.09 | 2873 | .. | .. | 66.197 | 15.561 | 10.530 | 9.698 | 99 | 5.049 |
| Av. per day | | 9.87 | 410 | .. | .. | 9.457 | 2.223 | 1.504 | 1.385 | .. | 0.721 |

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EXPERIMENT II. CÆSIUM PERIOD.

| Day of exp. | Body wt. | Food. | | Urine. | | | | | | | Fæces. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | CsCl. | Vol. | Spec. grav. | Reaction to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. |
| | kilos. | grams. | grams. | c.c. | | | grams. | grams. | grams. | grams. | gms. | grams. |
| 8 | 12.0 | 9.87 | 3 | 460 | 1.027 | acid | 10.603 | | 1.141 | 1.813 | | |
| 9 | 12.0 | 9.73 | 3 | 450 | 1.025 | " | 6.966 | | 1.582 | 1.861 | | |
| 10 | 11.9 | 9.73 | 3 | 415 | 1.027 | " | 9.598 | 9.555 | 1.118 | 2.053 | 71 | 3.621 |
| 11 | 11.8 | 9.73 | 3 | 445 | 1.024 | " | 8.298 | | 1.446 | 1.986 | | |
| 12 | 11.8 | 9.73 | 3 | 630 | 1.021 | " | 10.571 | | 1.288 | 1.861 | | |
| | | | | | N of urine . | | 46.036 | | | | | |
| | | | | | N of fæces . | | 3.621 | | | | | |
| Totals . . . | | 48.79 | 15 | 2400 | .. | .. | 49.657 | 9.555 | 6.575 | 9.574 | 71 | 3.621 |
| Av. per day | | 9.76 | 3 | 480 | .. | .. | 9.931 | 1.911 | 1.315 | 1.915 | .. | 0.722 |

EXPERIMENT II. SUMMARY, AVERAGE PER DAY.

| Periods. | Total Nitrogen. | | | Urine. | | | | | Fæces. |
|---|---|---|---|---|---|---|---|---|---|
| | Ingested. | Excreted. | Balance. | Vol. | Nitrogen. | $SO_3$. | $P_2O_5$. | Cl. | Nitrogen. |
| | grams. | grams. | gram. | c.c. | grams. | grams. | grams. | grams. | gram. |
| Fore | 9.87 | 9.457 | +0.413 | 410 | 8.734 | 2.223 | 1.504 | 1.385 | 0.721 |
| Cæsium | 9.76 | 9.931 | −0.171 | 480 | 9.207 | 1.911 | 1.315 | 1.915 | 0.722 |

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*Third experiment.* — In this experiment a bitch weighing six kilos was used. There were two periods, — a fore period of five days, and a cæsium period of three days. Approximate equilibrium was maintained on a diet of:

| | | |
|---|---|---|
| 125 | grams | meat, |
| 20 | " | lard, |
| 20 | " | cracker dust, |
| 100 | " | water. |

During the latter period a total of six grams was given, beginning with one gram on the first day, and increasing a gram each day. This amount caused no disturbance; but after the administration of four grams on the fourth day, there appeared gastro-intestinal disturbances so marked that further quantitative estimations of the urine were discontinued. The symptoms were similar to those shown by the preceding dog. No further excretion of the salt could be detected two weeks later. The nitrogen balance during the cæsium period was, if anything, slightly greater than during the fore period. There was no marked difference in the excretion of $SO_3$, $P_2O_5$, and Cl, except for the small quantities of the latter which were introduced with the cæsium.

EXPERIMENT III. FORE PERIOD.

| Day of exp. | Body wt. | FOOD. | URINE. | | | | | | | FÆCES. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | Vol. | Spec. grav. | Reaction to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. |
| | kilos. | grams. | c.c. | | | grams. | grams. | grams. | grams. | grams. | gram. |
| 1 | 6.0 | 4.856 | 182 | 1.028 | acid | 4.826 | | 0.954 | 1.178 | | |
| 2 | 6.0 | 4.856 | 165 | 1.029 | " | 4.794 | | 0.875 | 1.170 | | |
| 3 | 5.8 | 4.856 | 182 | 1.027 | " | 4.886 | 4.776 | 0.864 | 1.151 | 13.0 | 0.652 |
| 4 | 6.0 | 4.856 | 174 | 1.030 | " | 4.740 | | 0.802 | 1.450 | | |
| 5 | 5.8 | 4.786 | 190 | 1.028 | " | 5.034 | | 0.937 | 1.235 | | |
| | | | | N of urine | . | 24.280 | | | | | |
| | | | | N of fæces | . | 0.652 | | | | | |
| Totals . . . | | 24.210 | 893 | .. | .. | 24.932 | 4.776 | 4.432 | 6.184 | 13.0 | 0.652 |
| Av. per day | | 4.842 | 178 | .. | .. | 4.986 | 0.955 | 0.886 | 1.232 | .. | 0.130 |

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EXPERIMENT III. CÆSIUM PERIOD.

| Day of exp. | Body wt. | Food. | | Urine. | | | | | | | Fæces. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | CsCl. | Vol. | Spec. grav. | Re-action to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. |
| | kilos. | grams. | grams. | c.c. | | | grams. | grams. | grams. | grams. | gms. | gram. |
| 6 | 5.8 | 4.786 | 1.0 | 210 | 1.025 | acid | 4.748 | .... | 0.802 | 1.439 | | |
| 7 | 5.7 | 4.786 | 2.0 | 215 | 1.028 | " | 4.932 | 3.165 | 0.875 | 1.719 | 8.0 | 0.401 |
| 8 | 5.7 | 4.786 | 3.0 | 195 | 1.031 | " | 4.895 | .... | 0.898 | 1.765 | | |
| | | | | | N of urine . 14.575 | | | | | | | |
| | | | | | N of fæces . 0.401 | | | | | | | |
| Totals . . . | | 14.358 | .. | 620 | .. | .. | 14.976 | 3.165 | 2.575 | 4.932 | 8.0 | 0.401 |
| Av. per day | | 4.786 | .. | 207 | .. | .. | 4.992 | 1.055 | 0.858 | 1.641 | .. | 0.134 |

EXPERIMENT III. SUMMARY, AVERAGE PER DAY.

| Periods. | Total Nitrogen. | | | Urine. | | | | | Fæces. |
|---|---|---|---|---|---|---|---|---|---|
| | Ingested. | Excreted. | Balance. | Vol. | Nitrogen. | $SO_3$. | $P_2O_5$. | Cl. | Nitrogen. |
| | grams. | grams. | gram. | c.c. | grams. | grams. | gram. | grams. | gram. |
| Fore | 4.842 | 4.986 | - 0.144 | 178 | 4.856 | 0.955 | 0.886 | 1.237 | 0.130 |
| Cæsium | 4.786 | 4.992 | - 0.206 | 207 | 4.858 | 1.055 | 0.858 | 1.641 | 0.134 |

*Fourth experiment.* — About one month after the last experiment the same bitch was again brought into approximate equilibrium during a fore period of six days. The daily diet during this experiment consisted of:

135 grams meat,
20 " lard,
40 " cracker dust,
100 " water.

During the second period, one gram of the cæsium salt (about two hundred and ten milligrams per kilo body weight) was given daily for five days. The first four days brought out no unusual symptoms, the

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animal appearing well. On the fifth day, the animal seemed less well and had less appetite. The next morning the further feeding of the salt was abandoned, as the animal showed marked symptoms of its toxic action. Food was taken only after coaxing, and then most of it was vomited again. The fæces were watery and light in color. The animal was scarcely able to stand; the same evidence of loss of control being noted as has already been described, and the animal seemingly having lost the power of co-ordination for its hind legs. It was unable to hold the head erect. These symptoms continued to grow more marked until the next night, when death ensued, the animal being completely paralyzed. A few hours before death its reflexes were tested. There was noted the same progressive loss already mentioned in the injection experiments.

Until the marked toxic symptoms appeared, there was no difference in the metabolism of the two periods, as reference to the tables will show. The slight differences in nitrogen, $SO_3$, $P_2O_5$, and Cl are of no moment. The animal's weight was not greatly lessened. The experimental data are given in the table.

EXPERIMENT IV. FORE PERIOD.

| Day of exp. | Body wt. | FOOD. | URINE. | | | | | | | FÆCES. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | Vol. | Spec. grav. | Reaction to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. |
| | kilos. | grams. | c.c. | | | grams. | grams. | grams. | grams. | grams. | gram. |
| 1 | 4.8 | 5.255 | 177 | 1.027 | acid | 4.576 | 7.626 | 0.791 | 1.113 | 11.0 | 0.536 |
| 2 | 4.8 | 5.255 | 250 | 1.027 | " | 5.806 | | 0.904 | 1.362 | | |
| 3 | 4.8 | 5.647 | 227 | 1.025 | " | 4.934 | | 0.892 | 1.285 | | |
| 4 | 4.8 | 5.647 | 230 | 1.031 | " | 6.426 | | 0.881 | 1.285 | | |
| 5 | 4.8 | 5.647 | 240 | 1.029 | " | 5.471 | | 0.847 | 1.343 | | |
| 6 | 4.8 | 5.647 | 260 | 1.026 | " | 5.967 | | 0.898 | 1.247 | | |
| | | | | N of urine | . | 33.180 | | | | | |
| | | | | N of fæces | . | 0.536 | | | | | |
| Totals . . . | | 33.098 | 1384 | .... | .... | 33.716 | 7.626 | 5.213 | 7.635 | 11.0 | 0.536 |
| Av. per day | | 5.516 | 231 | .... | .... | 5.619 | 1.271 | 0.869 | 1.272 | .. | 0.089 |

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EXPERIMENT IV. CÆSIUM PERIOD.

| Day of exp. | Body wt. | Food. | | Urine. | | | | | | | | Fæces. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | CsCl. | Vol. | Spec. Grav. | Re-action to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. |
| | kilos. | grams. | grams. | c.c. | | | grams. | grams. | grams. | grams. | grams. | gram. |
| 7 | 4.7 | 5.647 | 1.0 | 230 | 1.027 | acid | 5.714 | | 0.847 | 1.362 | | |
| 8 | 4.6 | 5.647 | 1.0 | 220 | 1.030 | " | 5.691 | | 0.841 | 1.381 | | |
| 9 | 4.6 | 5.647 | 1.0 | 175 | 1.040 | " | 5.590 | 6.566 | 0.807 | 1.439 | 8.5 | 0.440 |
| 10 | 4.6 | 5.647 | 1.0 | 202 | 1.030 | " | 4.843 | | 0.819 | 1.458 | | |
| 11 | 4.6 | 5.647 | 1.0 | 160 | 1.031 | " | 4.880 | | 0.824 | 1.132 | | |
| | | | | | N of urine . | | 26.718 | | | | | |
| | | | | | N of fæces . | | 0.440 | | | | | |
| Totals . . . | | 28.235 | 5.0 | 987 | .... | .... | 27.158 | 6.566 | 4.138 | 6.772 | 8.5 | 0.440 |
| Av. per day | | 5.647 | 1.0 | 197 | .... | .... | 5.432 | 1.313 | 0 827 | 1.354 | .. | 0.088 |

EXPERIMENT IV. SUMMARY, AVERAGE PER DAY.

| Periods. | Total Nitrogen. | | | Urine. | | | | | Fæces. |
|---|---|---|---|---|---|---|---|---|---|
| | In-gested. | Ex-creted. | Balance. | Vol. | Nitrogen. | $SO_3$. | $P_2O_5$. | Cl. | Nitrogen. |
| | grams. | grams. | gram. | c.c. | grams. | grams. | grams. | grams. | gram. |
| Fore | 5.516 | 5.619 | −0.103 | 231 | 5.530 | 1.271 | 869 | 1.272 | 0.089 |
| Cæsium. | 5.647 | 5.432 | +0.215 | 197 | 5.343 | 1.313 | 827 | 1.354 | 0.088 |

*Fifth experiment.* — For this a bitch of seven kilos was used. The experiment lasted twenty-four days, consisting of a fore and after period of six days each, and a twelve-day cæsium period between these. Almost exact equilibrium was maintained during the fore period by feeding:

175 grams meat,
20 " lard,
40 " cracker dust,
150 " water,

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On the first eight days of the cæsium period one and four-tenths grams (two hundred milligrams per kilo body weight) of the salt were added to the food. For the next two days, one and three-quarters grams were given, — an increase to two hundred and fifty milligrams per kilo body weight. On the last two days of the period two hundred and seventy-five milligrams per kilo body weight were given amounting to one and nine-tenths grams. As the experiment progressed, there was only a slight change in the nitrogen balance. No toxic or unusual symptoms were noted, the only effect of the largest dose being a tendency to produce diarrhœa. The $SO_3$ excreted during the last two periods was slightly less than the output during the fore period, but this difference is too small to be of serious significance. The nitrogen and $P_2O_5$ output were practically unchanged, as was the Cl, — when the amount added to the diet in the cæsium period is taken into consideration. Cæsium was still being excreted three weeks after the last dose was fed. The experimental results are given in the tables:

EXPERIMENT V. FORE PERIOD.

| Day of exp. | Body weight. | Food. | Urine. | | | | | | | Fæces. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | Vol. | Spec. grav. | Reaction to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$ | Total Cl. | Dry wt. | Nitrogen. | Ether extract. |
| | kilos. | grams. | c.c. | | | grams. | grams. | grams. | grams. | gms. | grams. | per cent. |
| 1 | 7.0 | 6.903 | 250 | 1.029 | acid | 6.770 | | 0.955 | 0.921 | | | |
| 2 | 7.0 | 6.903 | 220 | 1.031 | " | 6.495 | | 1.018 | 1.036 | | | |
| 3 | 7.0 | 6.903 | 225 | 1.027 | " | 6.219 | 9.361 | 0.870 | 0.959 | 35 | 1.613 | 32.7 |
| 4 | 7.0 | 6.903 | 230 | 1.027 | " | 6.495 | | 0.978 | 1.017 | | | |
| 5 | 7.0 | 6.903 | 250 | 1.026 | " | 6.701 | | 0.983 | 0.940 | | | |
| 6 | 7.0 | 6.903 | 260 | 1.024 | " | 6.609 | | 0.927 | 0.978 | | | |
| | | | | N of urine . | | 39.389 | | | | | | |
| | | | | N of fæces . | | 1.613 | | | | | | |
| Totals . . | | 41.418 | 1435 | .. | .. | 41.002 | 9.361 | 5.731 | 5.851 | 35 | 1.613 | |
| Av. p. day | | 6.903 | 239 | .. | .. | 6.834 | 1.560 | 0.955 | 0.975 | .. | 0.268 | |

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EXPERIMENT V. CÆSIUM PERIOD.

| Day of exp. | Body weight. | Food. | | Urine. | | | | | | | Fæces. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen. | CsCl. | Vol. | Spec. grav. | Reaction to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Dry wt. | Nitrogen. | Ether extract. |
| | kilos. | grams. | grams. | c.c. | | | grams. | grams. | grams. | grams. | gms. | grams. | p. cent. |
| 7 | 7.0 | 6.903 | 1.4 | 260 | 1.028 | acid | 6.472 | | 0.915 | 1.343 | | | |
| 8 | 6.9 | 6.903 | 1.4 | 160 | 1.038 | " | 6.196 | | 0.966 | 1.151 | | | |
| 9 | 6.9 | 6.903 | 1.4 | 245 | 1.026 | " | 6.357 | | 0.841 | 1.285 | | | |
| 10 | 7.0 | 6.903 | 1.4 | 245 | 1.026 | " | 6.426 | | 0.904 | 1.151 | | | |
| 11 | 6.9 | 6.903 | 1.4 | 255 | 1.027 | " | 6.678 | | 0.989 | 1.381 | | | |
| 12 | 6.9 | 6.903 | 1.4 | 240 | 1.025 | " | 6.701 | 14.874 | 1.023 | 1.228 | 52.0 | 2.881 | 27.7 |
| 13 | 6.9 | 7.043 | 1.4 | 200 | 1.032 | " | 6.609 | | 0.910 | 1.228 | | | |
| 14 | 6.9 | 7.043 | 1.4 | 230 | 1.030 | " | 6.494 | | 0.893 | 1.305 | | | |
| 15 | 6.9 | 7.043 | 1.75 | 270 | 1.028 | " | 6.770 | | 0.955 | 1.285 | | | |
| 16 | 6.9 | 7.043 | 1.75 | 205 | 1.040 | " | 6.930 | | 1.012 | 1.208 | | | |
| 17 | 6.9 | 7.068 | 1.92 | 225 | 1.028 | " | 6.609 | | 0.995 | 1.362 | | | |
| 18 | 6.9 | 7.068 | 1.92 | 250 | 1.035 | " | 7.182 | | 1.026 | 1.420 | | | |
| | | | | | N of urine . | | 79.424 | | | | | | |
| | | | | | N of fæces . | | 2.881 | | | | | | |
| Totals . | | 83.726 | 11.84 | 2985 | .. | .. | 82.305 | 14.874 | 11.429 | 15.347 | 52.0 | 2.881 | |
| Average per day | | 6.977 | 1.54 | 248 | .. | .. | 6.862 | 1.239 | 0.952 | 1.278 | .. | 0.240 | |

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EXPERIMENT V. AFTER PERIOD.

| Day of exp. | Body weight. | Food. Nitrogen. | Urine. Vol. | Spec. grav. | Reaction to litmus. | Nitrogen. | Total $SO_3$. | Total $P_2O_5$. | Total Cl. | Fæces. Dry Wt. | Nitrogen. | Ether extract. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | kilos. | grams. | c.c. | | | grams. | grams. | grams. | grams. | gms. | grams. | per cent. |
| 19 | 6.8 | 7.068 | | | | | | | | | | |
| 20 | 6.8 | 7.068 | 230 | 1.035 | acid | 6.955 | 6.130 | 1.026 | 1.047 | 22 | 1.218 | 28.5 |
| 21 | 6.8 | 7.068 | 230 | 1.032 | " | 6.627 | | 1.012 | 0.894 | | | |
| 22 | 6.8 | 7.068 | 245 | 1.033 | " | 6.811 | | 0.944 | 1.009 | | | |
| 23 | 6.8 | 7.068 | 250 | 1.030 | " | 6651 | | 0.892 | 1.056 | | | |
| 24 | 6.8 | 4.233 | 193 | 1.027 | " | 4.131 | | 0.773 | 1.094 | | | |
| | | | | N of urine . | | 31.175 | | | | | | |
| | | | | N of fæces . | | 1.218 | | | | | | |
| Totals . . | | 32.505 | 1148 | .... | .... | 31.393 | 6.130 | 4.647 | 5.100 | 22 | 1.218 | |
| Av. p. day | | 6.417 | 229 | .... | .... | 6.278 | 1.226 | 0.929 | 1.020 | .. | 0.243 | |

EXPERIMENT V. SUMMARY, AVERAGE PER DAY.

| Periods. | Total Nitrogen. Ingested. | Excreted. | Balance. | Urine. Vol. | Nitrogen. | $SO_3$. | $P_2O_5$. | Cl. | Fæces. Nitrogen. |
|---|---|---|---|---|---|---|---|---|---|
| | grams. | grams. | gram. | c.c. | grams. | grams. | gram. | grams. | gram. |
| Fore | 6.903 | 6.834 | +0.069 | 239 | 6.565 | 1.560 | 0.955 | 0.975 | 0.268 |
| Cæsium | 6.977 | 6.862 | +0.115 | 248 | 6.619 | 1.239 | 0.952 | 1.278 | 0.240 |
| After | 6.417 | 6.278 | +0.139 | 229 | 6.235 | 1.226 | 0.929 | 1.020 | 0.243 |

**General considerations on metabolism.** — From the foregoing experiments it seems demonstrated that cæsium chloride in the doses given — forty to two hundred and seventy-five milligrams per kilo body weight — does not seriously affect proteid metabolism. When the dose becomes larger, a disturbing action on the gastro-intestinal tract results, as is evinced by vomiting and diarrhœa, with an occasional

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bloody fæcal discharge. A dose sufficient to produce even such toxic effects has no noticeable action on proteid metabolism. The nitrogen output was not altered; at least such changes as occurred were too slight to be of moment. In the first experiment there was a fairly large progressive change, but this is the only one showing such. In Experiment II, a plus balance of 0.413 gram changed during the cæsium period to a deficit of 0.171 gram. In Experiment V, the average daily nitrogen balance in grams for the three periods was respectively:

+0.069 +0.115 +0.139.

When larger amounts of the salt were given the balance was destroyed, because the animals refused food or vomited when fed by force. Under such experimental conditions, the intake of nitrogen could not be accurately determined, and estimations could not be made.

The results given agree in general with those reported by Krummacher[1] for sodium chloride (injected subcutaneously). Straub[2] also reported that feeding comparable amounts of sodium chloride did not alter the nitrogen output. Pugliese,[3] on the other hand, observed that this salt depressed proteid metabolism, whereas potassium chloride increased the exchange of nitrogen. Larger amounts of the salts were used than those fed in the present experiments.

The excretion of sulphur and phosphorus, as might be expected, ran parallel to that of nitrogen. There was no difference of any consequence in the various periods. No evidence of excessive phosphorus metabolism indicating marked nuclear or cellular decomposition was obtained. In this respect, the experiments agree with the results obtained by the observers just mentioned, after giving the chlorides of the other alkali metals.

During the cæsium period, the amount of chlorides eliminated increased to an extent about equal to the amount of chlorine added to the diet with the cæsium. Thus, in Experiment II, the animal received three grams of chlorine in excess of that in the food. All except thirty centigrams of this was eliminated during the same period. In the last experiment (V), a total of 3.9 grams of chlorine was added as cæsium chloride. During this period there was an excess eliminated over the first period of 3.6 grams; the after period

[1] KRUMMACHER: Zeitschrift für Biologie, 1900, xl, p. 173.
[2] STRAUB: Zeitschrift für Biologie, 1894, xxxvii, p. 545.
[3] PUGLIESE: Archives italiennes de biologie, 1896, xxv, p. 17.

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contained an excess over the fore period of 0.2 gram chlorine. The chloride apparently is not stored up to any extent, but is for the most part rapidly eliminated. Cæsium could often be detected spectroscopically for several days after its administration. It must be remembered, however, that this test is a very delicate one.

In those experiments (III, IV, V,) in which the daily portions of urine were carefully separated by catheterization, the elimination of water by the kidneys was practically constant throughout the feeding. No noticeable disturbing effect was obtained even with the largest doses recorded. Pugliese reported similar results after feeding the chlorides of sodium and potassium.

**The elimination of cæsium.** — Cæsium is excreted by way of the intestine as well as through the kidneys. Its elimination follows quite rapidly, although traces could be demonstrated spectroscopically in the urine of some animals for many days after the last feeding. The salt disappeared from the fæces before its presence ceased to be demonstrable in the urine. Whether this is attributable to the greater ease with which minute traces can be detected in the urine, or whether, as a matter of fact, the last traces are eliminated by way of the kidneys is not certain. In those cases in which the organs and tissues were carefully examined spectroscopically at a period when cæsium ceased to be demonstrable in the urine, no evidence of the element was obtained. When various parts of the alimentary tract were examined spectroscopically after subcutaneous or intravenous injection of cæsium chloride, cæsium was detected throughout the entire length of the intestine. In the stomach, the reaction was usually less pronounced. If we recall that cæsium was shown above to be excreted by the salivary glands under such circumstances, it may be questioned whether the reaction obtained with the gastric contents was not in part or entirely due to admixture of saliva. Functional differences in the secretory processes along the digestive tract have been shown to exist in the case of various other substances similarly examined. Portions of hair from some of the animals which had received doses of cæsium for some time were examined spectroscopically with negative results.

A few experiments on the elimination of cæsium were carried out on man. The individuals took doses varying from fifty to three hundred and seventy-five milligrams dissolved in one hundred cubic centimetres of water in each case. Only with the largest dose was cæsium detected in the urine within the period of observation. In

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this instance the spectroscopic reaction was evident at the end of an hour. The fæces were not examined. The observations of Hüfner[1] on the excretion of lithium in man are of interest in this connection. The elimination of cæsium through the intestine is interesting in view of the fact that relatively few elements (Ca, Mn, Fe, Li) have been shown to be excreted by that channel. Its presence in the saliva recalls the work of Bernard,[2] Langley and Fletcher,[3] and Eckhard,[4] with potassium iodide, lithium citrate, and other salts. These investigators have made it clear that not all salts introduced into the circulation reappear in the saliva. Certain of these, such as the ferrocyanides, fail in this respect. For lithium Good[5] has recently found in experiments on cats that when large quantities (one to two grams) were injected hypodermically, very considerable amounts were obtained from the stomach (by lavage), and from the bowel; and the saliva also contained appreciable quantities. In fatal poisoning, more was found in the stomach and bowel contents than in the urine. In experiments in which small doses were administered repeatedly, more lithium was excreted by the urine than by the alimentary tract.

## Summary.

*Experiments on frogs.* — 1. No marked differences between the effect of $\frac{n}{8}$ solutions of sodium chloride and cæsium chloride upon cilia and red blood-corpuscles were noted.

2. Isolated muscles contract spontaneously in such solutions. The contractions cease sooner in the cæsium than in the sodium solution.

3. Muscles exposed to cæsium chloride lose their irritability toward electrical stimulation sooner than those exposed to sodium chloride.

4. The irritability of nerves disappears sooner in cæsium chloride than in sodium chloride solutions.

5. Complete paralysis is produced by considerable doses of cæsium chloride (one to two and one-half milligrams per gram body weight).

[1] Hüfner: Zeitschrift für physiologische Chemie, 1880, iv, p. 378.

[2] Bernard: Leçons de physiologie expérimentale, 1856, ii.

[3] Langley and Fletcher: Philosophical transactions of the Royal Society of London, 1889, clxxx, p. 149.

[4] Eckhard: Beiträge zur Physiologie, Leipzig, 1887, p. 13. Quoted in Schäfer's Text-book of physiology, i, p. 504.

[5] Good: This journal, 1902, vi, p. xx; American journal of the medical sciences, 1903, cxxv, p. 273. All of these observations on lithium are of interest in comparison with those noted in this paper.

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6. No initial excitability was noted.

7. The cæsium salt seems to act on both nerves and muscles.

*Experiments on mammals.* — 8. In the rabbit, cat, and dog, final paralysis followed the subcutaneous injection of cæsium chloride.

9. Two grams per kilo body weight (subcutaneously) proved fatal to a rabbit. One-half to one gram per kilo body weight usually proved fatal to a cat or dog.

10. The symptoms elicited were those of intense gastro-intestinal disturbance, vomiting, diarrhœa, loss of reflexes, and progressive paralysis.

11. Intravenous injection produced an initial fall, followed by a marked rise in blood-pressure in both cats and dogs. The lymph flow from the thoracic duct was slightly accelerated.

12. Fatal doses (about three-quarters of a gram per kilo body weight) caused death by cardiac failure.

13. Proteid metabolism in dogs was not noticeably disturbed by doses of from forty to two hundred and seventy-five milligrams per kilo body weight fed for several days. Sulphur and phosphorus metabolism were also unaffected. Chlorine elimination was unchanged, except that the added amount fed with the cæsium was rapidly excreted.

14. No diuretic action was obtained.

15. When larger amounts were given per os, marked gastro-intestinal disturbances resulted.

16. Elimination of the cæsium by the intestine and kidneys was comparatively rapid, no prolonged retention being noted.

17. In man, the cæsium was detected in the urine within an hour after ingestion of three hundred and seventy-five milligrams of cæsium chloride.

I desire to express my obligation to Professor Chittenden for suggesting this research, and to Professor Lafayette B. Mendel for help and criticism.

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# SOME FACTS CONCERNING GEOTROPIC GATHERINGS OF PARAMECIA.

By ANNE MOORE.

## Introduction.

In experimenting with Paramecia it is often found necessary as a preliminary measure to wash them in distilled water. To accomplish this it has been customary to take advantage of the fact that if they are removed from the culture to a test tube of distilled water they will, after swimming about actively, come to rest at the top of the tube. In following this method, I noticed some peculiarities in their behavior which seemed to me worth recording and worth further experimentation. The gathering at the top was apparently an expression of negative geotropism.[1] I wished to ascertain whether, having once gathered at the top, the Paramecia would change their position to the bottom of the tube, or, in other words, whether the sense of geotropism ever changes normally from negative to positive, and, if so, to ascertain what factors influence the change.

The experiments were begun in the Physiological Laboratory of the University of Chicago, and were afterward continued in San Diego, California.

## Observations at Room Temperature (18°–20° C.).

Small glass tubes half a centimetre in diameter and twelve centimetres long were used. These were filled to within two centimetres of the top with distilled water, and then completely filled with water from the culture containing Paramecia. The Paramecia were at first carried downward with the stream of water, but soon began to swim about independently. Although their orientation was not uniform, there was a more or less steady movement downward. When the bottom was reached, which occurred usually in about fifteen minutes,

[1] Although the gathering at the top seemed not always to be the direct effect of an orientation, the word geotropism has been used for convenience, as indicative of the general character of the phenomena.



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they remained massed there a few moments, and then formed about the wall of the tube a well defined ring. This ring then began to rise slowly, the individuals composing it remaining massed close against the glass and against each other, indicating a stereotropic reaction. Occasionally the ring was thicker on the light side of the tube than on the other side, but this was not invariably true. The upward movement of the ring seemed independent of active swimming and of definite orientation. The rapidity with which the ring rose depended somewhat upon the temperature; the lower the temperature the more slowly rising occurred. Ordinarily the minimum time in which it might be expected to reach the top was about two hours. If the tube was left for several days after the top was reached, the Paramecia became scattered throughout the tube and finally collected at the bottom. It sometimes happened that the Paramecia, instead of behaving with one accord in this way, became divided into two groups, one of which remained at the bottom, while the other rose to the top, as described above.

As the rate of rising varies with the temperature, changes in temperature must be an important factor in determining the position of the animals, but as the sense of geotropism is not the same in all animals taken at the same time from the culture, and as it does not remain constant when external conditions are apparently unchanged, changes of temperature alone are insufficient to account for this behavior.

### The Effect of Changes of Temperature.

In Chicago, the experiments were tried during the winter. Several tubes were left for three hours in a thermostat at a temperature of 26°–28° C. The Paramecia collected at the top in dense clusters. On being removed from the thermostat to room temperature, they immediately streamed toward the bottom. They were returned to the thermostat, and left over night. The next morning they were found at the top, and, on being removed, again streamed downward. The tubes were put outside the window at a temperature of 2° C. At first the Paramecia scattered, but in ten minutes they were all at the bottom of the tubes. One tube was returned to the thermostat. Fifteen minutes later a ring had formed, and had begun to rise, while in the tubes outside the window there were no signs of rising. If left outside, or in the thermostat for as long as two days, the Paramecia became scattered.

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These experiments were afterward repeated in San Diego, California, and results were obtained in harmony with those just described. The following experiment is typical. Two tubes were similarly prepared. Tube A was placed in a thermostat at a temperature of 26°–28° C.; tube B was placed in a larger tube filled with water and surrounded by a mixture of ice and salt, the temperature being kept as nearly as possible at 1° C. In ten minutes the Paramecia in tube B were massed at the bottom, and two hours later were still massed there. The tube was then transferred to the thermostat. The ring formed as usual and rose steadily. In an hour, however, the Paramecia scattered throughout the tube. If scattering occurred, as a rule no ring was again formed until conditions were again changed. Consequently a quarter of an hour later the tube was removed to room temperature, 18° C. They remained scattered for a time, and then collected in two groups, one at the top, the other at the bottom of the tube. They were then returned to the thermostat, and an hour later were collected at the top. In tube A a ring had formed in ten minutes, and had begun to rise. In half an hour some of the Paramecia were at the top, but many were scattered. When returned to a temperature of 1° C., the process as described above occurred. The scattering of the Paramecia after having been made to gather at the top or bottom by an unusual temperature is an interesting adjustment to environment, and suggests the observations of Yasuda,[1] who found that protozoa have a remarkable power of adapting themselves to changes in the osmotic pressure of the medium surrounding them. It suggests also the observations of Jennings,[2] who shows that Infusoria, after reacting to a given stimulus one or more times, may, if the stimulus is not a harmful one, cease to react though the stimulus is repeated without change. Without attempting an explanation, he suggests that there is a difference in the physiological condition of the organism before and after the stimulus. The inversion of the sense of geotropism indicated in the experiments described above is a further illustration of the principle pointed out by Loeb,[3] that by appropriate stimulation the sense of an animal's response may be inverted. He showed that changes in heliotropism may be correlated with changes in temperature. Increase

[1] YASUDA, ATSUSHI: Journal of the College of Science, Imperial University, Tokyo, 1900, xiii, p. 101.

[2] JENNINGS, H. S.: This journal, 1902, viii, p. 23.

[3] LOEB, J.: Archiv für die gesammte Physiologie, 1893, liv, p. 81.

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in temperature, for example, causes the Copepod Temora longicornis to change its sense of heliotropism from positive to negative; while decrease in temperature causes the change from negative to positive.

## The Effect of Withdrawing Water.

An analogy has been noted between the effect of a lowering of the temperature and of the withdrawal of water. Loeb,[1] for example, showed that the larvæ of Polygordius and certain Copepods can be made positively heliotropic by increasing the concentration of the sea-water or by decreasing the temperature, and can be made negative by decreasing the concentration of the sea-water or by raising the temperature. He therefore came to the conclusion that raising the concentration of the salt solution has qualitatively and quantitatively the same effect as lowering the temperature. Greeley[2] has recently called attention to the fact that this is true, because the cell loses water when the temperature is lowered, as well as when the concentration of the surrounding medium is changed. I therefore tried the effect of weak salt solutions upon the behavior of Paramecia. The results were, however, not very satisfactory. When first put into $\frac{n}{32}$ NaCl, or into weaker solutions of sodium chloride, there was a general scattering as in water, but after an hour they gathered more thickly near the top. After several days they showed a tendency to go to the bottom. In $\frac{n}{16}$ NaCl, they went to the bottom almost immediately, but shortly after died. In a solution of calcium chloride isosmotic with $\frac{n}{32}$ NaCl, they went immediately to the bottom. In this solution water is withdrawn very readily.

In accordance with a suggestion of Davenport, Miss Platt[3] approached the problem of geotropism (geotaxis) by means of varying solutions, in order to test a conclusion reached by Schwarz that negative geotropism is due to "the direct influence of gravity on the organism which incites movement in the opposite direction." Her plan was to obtain the specific gravity of the organism "by finding the density of a solution in which the animals, either dead or paralyzed, remained suspended without rising or falling," and then to observe their movements in solutions of varying density. For if geotropism were a weight effect, a negatively geotropic organism should become

[1] Loeb: *Loc. cit.*

[2] Greeley: This journal, 1901, vi, p. 122.

[3] Platt, Julia B.: The American Naturalist, 1899, xxxiii, p. 31.

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positively geotropic in solutions of greater specific gravity than its own, supposing the animal to be normally heavier than water. She found the specific gravity of Paramecia to be approximately 1.017, but she could come to no conclusion from its behavior, for in Cambridge she could find no Paramecia with decided geotropic tendencies.

From experiments on tadpoles, however, she concludes that the direct action of gravity, as expressed in the weight of the organisms, does not act as the incentive to negative geotropism, and she suggests that it is possible, both in tadpoles and in infusoria, that gravity may act through the internal organization of the animal, and that in this case the density of the surrounding medium might be expected to effect little if any change in the direction in which the organism moves. And indeed I find this to be true. In solutions of gum arabic of greater specific gravity than the Paramecia, the animals remain at the top or become scattered. If, however, changes in density of the surrounding medium are such as affect materially the amount of water in the protoplasm, as the movements of the organism are ultimately dependent upon the organization of the protoplasm, *a priori*, one would expect such changes to affect such reactions as the negative response to gravity. In salt solutions, therefore, in which water is withdrawn, the internal constitution of their protoplasm is so altered that the Paramecia go to the bottom.

## Influence of Food.

The fact that Paramecia were found at the bottom of the tube after remaining several days in $\frac{n}{32}$ NaCl may be not entirely due to the withdrawal of water. It was found that when Paramecia were left in a tube of distilled water for some time, without the addition of food, they finally collected at the bottom and remained there. If water containing food was added, they were found next morning in a ring at the top of the tube. This indicates that the sense of geotropism differs in Paramecia when deprived of food and when food is supplied in abundance. It suggests a case mentioned by Loeb.[1] The young caterpillars of Porthesia chrysorrhœa are positively heliotropic when hungry, but lose the sense of heliotropism when fed.

[1] Loeb: Comparative physiology of the brain, 1901, p. 195.

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### THE EFFECT OF MECHANICAL SHOCK.

It was mentioned above that when the tube was lifted from the thermostat the Paramecia streamed downward. This was noted on several occasions, and was probably not due simply to a change from the temperature 28° to the temperature 20°. Whether the Paramecia were in a ring, or whether they were scattered throughout the tube, it often happened that merely lifting the tube seemed to disturb them sufficiently to cause a downward movement. This suggested the idea that a more violent shock would cause a more marked gathering at the bottom of the tube. The tubes were therefore shaken by hand, with the unfailing result that the Paramecia collected at the bottom in a mass much more closely aggregated than was noted under any other circumstances, unless perhaps where the temperature was lowered to 1°.

This effect of a mechanical shock is by no means new. Loeb[1] showed that shaking made certain Copepods temporarily positive, and indeed agitation of the water has been one of the most successful means of producing an inversion of the sense of an animal's response. Towle[2] showed that even so slight a shock as contact with the mouth of a pipette was sufficient to render Cypridopsis, which is usually negatively heliotropic, temporarily positive. In spite of this, however, it seems well to emphasize here the effect of mechanical shock upon the geotropism of Paramecia, as it adds to the list of evidences of the importance of mechanical shock in the life history of body and other cells which of late have been forthcoming.[3] In a recent article by Matthews and Whitcher,[4] the statement is made that the most probable explanation of the action of mechanical shock on the egg-substance is that it "causes a partial gelation of the colloids of the egg-substance. It produces the same effect on the protoplasm as cold, and the two processes accordingly supplement each other. This conclusion is, we believe, strengthened by Mrs. Andrews's observations on living proto-

[1] LOEB, J.: Archiv für die gesammte Physiologie, 1893, liv, p. 96.

[2] TOWLE, E. W.: This journal, 1900, iii, p. 345.

[3] For example, in the work of Meltzer on the Influence of Agitation on Animal Cells and Bacteria, Zeitschrift für Biologie, 1894, xxx, p. 3; of MATTHEWS on Artificial Parthenogenesis in the Egg of the Starfish, This journal, 1901, vi, p. 142; and of FISCHER on Artificial Parthenogenesis in the Egg of Amphitrite, This journal, 1902, vii, p. 301.

[4] MATTHEWS and WHITCHER: This journal, 1903, viii, p. 300.

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plasm. She observed that a mechanical shock caused a distinct change in the viscidity of the choano-flagellates, a very small jar causing the collar to become rigid." In her article[1] Mrs. Andrews shows that the "viscosity of protoplasm varies locally very swiftly. From a very fluid state, it becomes rapidly so viscous as to resist pressure, after the manner of a stiff bristle." It would be strange indeed if such changes in the viscidity of the protoplasm were not accompanied by changes in the reactions to external stimuli of such animals as Paramecia.

## Summary.

1. Paramecia are sometimes positively geotropic, sometimes negatively geotropic, sometimes without the sense of geotropism.

2. Positive geotropism may be induced in Paramecia by

(*a*) Mechanical shock. The effect of the shock takes place quickly and is lost quickly.

(*b*) Reduction of temperature. At 1° C. the effect is marked, and takes place quickly. In a comparatively short time, the animals may adapt themselves to the low temperature and lose the sense of geotropism.

(*c*) Increase in concentration of the surrounding medium. This factor is not so constant as the other factors, and the effect not so marked.

(*d*) Lack of food. The effect takes place slowly and is lost slowly.

3. Negative geotropism may be induced in Paramecia conversely by a plentiful food supply and by an increase in temperature within limits.

4. The geotropic reactions of Paramecia to these influences is of importance in their life history, for, as the positive reaction carries them away from the surface, they would be protected from surface agitations, from the effect of surface ice, and from the failure of surface food-supply.

[1] Andrews: Journal of morphology (supplement), 1897, xii, p. 27.

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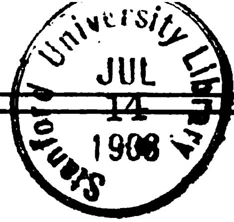


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# SOME OBSERVATIONS ON THE EFFECTS OF AGITATION UPON ARBACIA EGGS.

By S. J. MELTZER.

[*From the Marine Biological Laboratory, Woods Hole, Massachusetts.*]

FOR nearly twenty years I have studied at different times the mechanical effect of shaking on unicellular organisms. While spending my vacation last summer in Woods Hole and enjoying the hospitality of the Marine Biological Laboratory, I was anxious to study the effect of shaking upon the eggs of lower marine animals. Loeb[1] discovered that the simple increase in the osmotic pressure of the sea-water is sufficient to start the process of segmentation in unfertilized Arbacia eggs. Mathews[2] has shown that in unfertilized star-fish eggs this artificial parthenogenesis can be brought on by the mechanical effect of squirting the eggs with a pipette. Similar observations were made by Fischer[3] on the eggs of Amphitrite. In Arbacia eggs no artificial parthenogenesis could be started by the simple mechanical procedures employed by Mathews and Fischer.[4] In my studies of the effect of shaking upon bacteria[5] I found that different organisms differed in their behavior towards a certain degree of vibration, that a degree of shaking favorable to one organism might be indifferent to a second, and destructive to a third. As the very mild mechanical action employed by Mathews proved to be indifferent to the unfertilized Arbacia eggs, the question arose whether other or more energetic methods of shaking would not prove to be effective in one or the other direction upon the unfertilized as well as upon the fertilized eggs of the sea-urchin. The methods I have employed in my previous investigations upon blood-cells, as well as upon bacteria, were principally of two kinds. The organisms were either subjected to the finer continuous vibrations which exist

[1] LOEB: This journal, 1900, iii, p. 135.

[2] MATHEWS: This journal, 1901, vi, p. 142.

[3] FISCHER: This journal, 1902, vii, p. 301.

[4] LOEB: Archiv für Entwickelungsmechanik, 1901–02, xiii, p. 481.

[5] MELTZER: Zeitschrift für Biologie, 1894, xxx, p. 3.



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in the neighborhood of strong rhythmical shocks, for instance, in all parts of a building where large engines are continuously at work; or they were subjected to the direct effect of the shocks, produced by energetically shaking bottles, containing the organisms, to and fro by the hand or by machines. In this latter method the effect proved to be incomparably greater when small, corpuscular firm bodies were placed in the liquids within the bottles, — glass beads, for instance.

In the present series I have omitted the method of shaking gently by hand without the addition of other foreign bodies, since I found that Mathews and Whitcher[1] had started work on these lines. I confined myself to the study of the effects of more violent methods of shaking. The bottles which were shaken by free hand contained, besides the eggs, some glass beads, about one-tenth of the volume of the sea-water, the latter filling about one-third of the bottle. The shaking was carried on by not less than one hundred and eighty movements in a minute, mostly, however, two hundred and forty times and more in one minute. For other methods of shaking I have availed myself of the steam-engine and the dynamo located on the grounds of the United States Fish Commission. Bottles with or without beads were attached either to the piston of the steam-engine, which made only thirty-six to fifty excursions in a minute, or to the piston of the dynamo, which made steadily three hundred and sixty movements in a minute. Bottles containing Arbacia eggs were also placed on the large iron box of the steam-engine, or they were attached to a high pole connected with this box. In either case the eggs were subjected to a nearly continuous vibration emanating from the distant shock; the vibration was more intense on the pole than on the box, as could easily be felt and seen. The strongest effect of the direct shock was of course produced by the movements of the piston of the dynamo. The next strong effect was produced by shaking the bottles by the hand. The mildest effect of the direct shock was produced in the bottles attached to the piston of the steam-engine on account of the slowness of the movements of the latter. The temperature in the places where the bottles were attached was only slightly higher than that of the air.

I shall now report briefly some of the observations I thus gathered.

1. Among unfertilized Arbacia eggs, which had been subjected to any of the methods of shaking (except of course when attached to

[1] MATHEWS and WHITCHER: This journal, 1903, viii, p. 301.

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the piston of the dynamo), there were always a small number segmented, while segmented eggs were absent in most of the controls. The segmented eggs were mostly in the two and four cell stages, rarely in the eight-cell stage, and never in a more advanced stage. The usual precautions were taken to avoid contamination with sperm; this nevertheless does not absolutely exclude the possibility of an occasional contamination. However, it seems to me that the very fact that only a few eggs segmented, and that the segmentation did not advance beyond the very earliest stages, speaks against the possibility that the segmentation was due to a real fertilization which accidentally always could have occurred in the shaken specimen and rarely in the control.

Moreover, since it has been established that in the eggs of the star-fish and Amphitrite the entire series of segmentation can be started and brought to a finish by mechanical effects, it is surely nothing unusual to find that strong mechanical effects can induce some degree of cleavage, even in a few of the Arbacia eggs, though the latter are in general very little susceptible to artificial mechanical influences.

2. Shaking by hand was carried on with fertilized as well as unfertilized eggs for half a minute, one minute, two, three, four, and five minutes. The predominant feature of the effect of this mode of violent shaking was a greater or less destruction of the eggs. One interesting result came out very distinctly in every experiment: it is the fact of the superior resistance of the fertilized over the unfertilized eggs. The little bottles containing the fertilized or the unfertilized eggs were prepared exactly alike, and in every experiment a bottle of each kind was kept in the same hand, and shaken simultaneously for the same length of time. When examined under the microscope, the fertilized eggs shaken for five minutes looked, with regard to the number of disorganized eggs, like the unfertilized eggs shaken only for two minutes; or fertilized eggs shaken for three minutes looked like unfertilized, shaken for one minute and less. Furthermore, in the progress of the destruction of the unfertilized eggs, parallel with the disappearance of the eggs, there is a steady increase of dust-like débris. There are very few shapeless eggs or masses of large fragments present in the destroyed unfertilized eggs.

On the other hand, in the violently shaken fertilized eggs, we see plenty of disorganized eggs of all shapes, and coarse fragments of all sizes and forms, and see but little fine, dust-like débris.

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Unfertilized eggs are apparently more brittle; they offer less resistance to a mechanical shock, and break down more readily into dust; while the fertilized eggs are more elastic and coherent. I wish, however, to state expressly that by using the terms brittle and elastic, I do not mean to offer an hypothesis as to the real nature of the change of structure; these terms are only other expressions for the facts I have observed. What I would be inclined to insist upon, is that the change is of a physical character. Possibly the assumption of Mathews and Whitcher,[1] that the difference in the resistance is due to a change in the viscosity of the protoplasm, is correct. There are, however, many other possible interpretations of the facts which Mathews and Whitcher and myself have observed. Thus we may, for instance, assume that the physiological molecules of the eggs, which are stirred through the action of the sperm, and get into readiness to enter into a new grouping so as to facilitate segmentation, are more separated from one another. It is possible, therefore, that after fertilization each physiological molecule is surrounded by a greater sphere of an elastic fluid than before, and it is the elasticity of these spheres which acts as a buffer and dampens the effect of the mechanical shock.

We should not omit to add that Morgan[2] in his studies on the effect of centrifugalization upon eggs, found that it is more destructive to unfertilized than upon fertilized eggs.

In experiments on unfertilized eggs, it appeared further, that the longer the eggs were removed from the ovaries and preserved in sea-water, the less was their resistance toward mechanical shocks. However, no detailed studies were made on this point.

When unfertilized eggs were shaken for one minute, a small number of eggs remained uninjured, and a few have shown cleavage. In many experiments, the mass contained bodies smaller than eggs; they looked like small blastulæ, and some of them were swimming rapidly. Whether they were real artificial blastulæ, or were foreign invaders,[3] etc., I was unable to determine. When the unfertilized eggs were shaken three to five minutes, there were neither unsegmented nor segmented eggs, nor any swimming bodies to be seen.

Fertilized eggs, when shaken for one minute, even very soon after fertilization, appeared very little injured; there were hardly any

[1] MATHEWS and WHITCHER: *Loc. cit.*

[2] MORGAN: Archiv für Entwickelungsmechanik, 1902, xv, p. 238.

[3] See LOEB: Archiv für Entwickelungsmechanik, 1902, xiv, p. 288.

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fragments to be seen. But there were present quite a number of segmented eggs in an advanced stage, and even a few distinct blastulæ were seen. When shaken for five minutes, a good many injured eggs and large fragments were present, but the majority of the eggs were still intact. Among them there were many segmented eggs in all stages of cleavage.

Fertiiized eggs shaken for five minutes (by hand over two hundred times in a minute and with the addition of glass beads) contained blastulæ resting on the bottom of the vessel and a fair number of swimming gastrulæ, while the control contained nothing of that kind. The fact that by violent agitation of fertilized eggs the cycle of segmentation could be so hastened as to reach the gastrula stage in a few minutes, is so unusual that now, writing from notes and not being in a position to verify it, I hesitate to set it down unreservedly. When the eggs thus shaken are left alone, a good many more develop into blastulæ, but few into swimming gastrulæ, and none into plutei. All soon undergo decomposition.

Violent shaking of the eggs within the bottles attached to the piston of the dynamo thoroughly destroyed fertilized and unfertilized eggs alike in the shortest time.

Fertilized eggs which were kept in bottles on the box of the steam-engine, or were attached to the pole, and thus were subjected to a moderate but nearly continuous vibration, have been influenced in a double way. Eggs which were kept there from forty to eighty minutes have shown in all instances a marked advance in the segmentation over the eggs of the control. When in the control the four-cell stage predominated, with only a few of the eight-cell stage among them, the eggs subjected to vibration contained at this time predominantly eight or sixteen cell stages, or had progressed even farther. Furthermore, the segmentation of the eggs in the bottles attached to the pole (more vibration) was greater than that on the box, although the temperature on the pole was lower than on the box.

Keeping the eggs in the mentioned places for longer than two hours begins to affect their further development unfavorably, and the longer the eggs were subjected to the vibration, the more distinctly they remained behind the control. The stage of plutei, if it was ever attained, was reached ten or twelve hours later, all being small and mostly crippled. The shaken eggs, as a rule, had only few swimming gastrulæ, most of them were lying on the bottom. Even these, however, were, without exception, far in advance of those

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eggs which were shaken for a longer or shorter time, and then kept under the same conditions as the control. For instance, thirty-six hours or two days after the beginning of an experiment, while the control would be teeming with well-developed plutei, the eggs shaken for twenty-four hours would show a few crippled plutei, some swimming gastrulæ, a good many resting blastulæ and eggs in all stages of division ; the eggs which were made to vibrate for only one hour, and placed near the control, than which they were then more developed, would be already decomposed and emitting a foul odor.

In other words, if fertilized eggs were once subjected to artificial vibration, they became decomposed early, if the vibration were not continued.

The last series of experiments brings out the following instructive point. Mild vibrations of comparatively short duration, though they affect somewhat favorably the first steps of segmentation of fertilized eggs, upset their normal equilibrium to such a degree that further development is almost checked, unless vibration be continued, and even then the development of the eggs ultimately lags far behind that of the uninfluenced control eggs.

Our experiments have shown that any kind of shaking might start the first steps of cleavage in a few of the unfertilized Arbacia eggs. Our experiments with violent shaking by hand have shown that some fertilized or unfertilized Arbacia eggs might by violent agitation be hastened in a very short time into an advanced stage of cleavage. Finally, our experiments with mild vibrations have shown that the first steps in segmentation of fertilized eggs can be slightly hastened by these vibrations, but the experiments have also shown that these vibrations are incapable of bringing the development of the egg to a normal finish, and that they upset the normal equilibrium of the fertilized eggs.

Shaking or vibration, at least in the methods we employed, has proved to be incapable of producing artificial parthenogenesis in Arbacia eggs. But the experiments have shown that even these inadequate vibrations are capable of profoundly influencing the finer mechanism, underlying the process of segmentation, and that they do not simply injure the eggs in a coarse, traumatic manner.

In unfertilized Arbacia eggs, cleavage can be brought about by change in osmotic pressure, but not by simple mechanical means. In star-fish eggs, cleavage can be induced by very simple mechanical shocks, but not by the change in osmotic pressure. Since the dis-

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covery by Loeb of artificial parthenogenesis by the addition of chemicals to the sea-water, it has been often stated that the entrance of the spermatozoon into the ovum causes changes which might be similar in character to those which are caused by an alteration in the osmotic pressure of the suspension fluid. But why not also assume that the great motility of the sperm is in every case an adequate mechanical shock appropriate to start the normal cycle of cleavage, just as we see that a simple mechanical shock may be the means of setting up an artificial segmentation of the eggs of the star-fish? The artificial mechanical shocks at our disposal are capable of affecting the eggs profoundly, but for the purpose of setting up a nearly regular cleavage they are adequate only for eggs of one or the other species. Might it not be that the motility of the sperm is specific, and therefore adequate and effective in each special case? Perhaps a study of the different rates, characters, etc., of the motions of the different spermatozoa will throw some light upon this question.

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# ON THE EFFECTS OF SUBCUTANEOUS INJECTION OF THE EXTRACT OF THE SUPRARENAL CAPSULE UPON THE BLOOD-VESSELS OF THE RABBIT'S EAR.

By S. J. MELTZER and CLARA MELTZER.[1]

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## Effects upon the Ears of Normal Rabbits.

IN a previous communication[2] we recorded the observation that after an intravenous injection of adrenalin, the vasoconstriction in a rabbit's ear (on the non-operated side) is usually followed by a vasodilatation exceeding that which existed before the injection. A search in the literature on the subject satisfied us that our experience of the vasodilating after-effect is not an isolated one. Langley,[3] for instance, records that the submaxillary gland appears flushed after the blanching of the gland, caused by injection of suprarenal extract, vanishes. Regarding the general blood-pressure, Lewandowsky[4] states that in cats it frequently sinks below the original level, after the usual primary rise.

We have interpreted our observation by the assumption that the reduced dose of adrenalin within the blood stimulates preferably the central vasodilating mechanisms. On the basis of this hypothesis we started out to determine a small dose of adrenalin which by intravenous injection might be capable of causing a primary dilatation of the ear-vessels. As we have stated in the above-mentioned paper, we met with full success in only very few instances. In most of these experiments an injection of the small dose brought either no change at all, or there was no change for a minute or two, and then a perceptible dilatation followed, or finally there was a very brief primary constriction, followed by a pronounced dilatation of the vessels.

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[2] S. J. and Clara Meltzer: This journal, 1903, ix, p. 147.

[3] Langley, J. N.: Journal of physiology, 1901–1902, xxviii, p. 237.

[4] Lewandowsky: Archiv für Physiologie, 1899, p. 360.



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In a new series of experiments we studied the effect of adrenalin upon the blood-vessels in the ear of the normal rabbit, when introduced subcutaneously. These studies brought to light more clearly the vasodilating effect of the suprarenal extract. We shall report our results briefly.

These studies are not as easy a task as might appear at first thought. The blood-vessels of the rabbit's ear, as is well known, show a continual change in their volume. But these changes are not as regular as might appear from the statements of Schiff,[1] who was the first to observe them. Indeed, Becke van der Callenfels[2] published tables showing that there may be great irregularity in these changes. We can confirm his statements. Sometimes, indeed, constrictions of long duration are interrupted by short dilatations, as Schiff reported; but at times the dilatations last long and the constrictions are short, or both are short, or both long. The duration of each phase can vary from a few seconds to many minutes. It is obvious, therefore, that it is very difficult to say whether a dilatation of long duration which appears after an injection of adrenalin, is due to the injection or is one of the normal irregularities; or whether it is due to some of the numerous accidental conditions which favor dilatation. Warmth, for instance, favors dilatation. Thus dilatations prevailed in animals, the ears of which were observed by lamplight. Simple holding of the ears for the sake of better observation favors dilatation. Any motion of the animal causes paling of the ear, which is then followed by a long lasting flush. There is also an individual variability; in some rabbits constrictions, in others dilatations, preponderate. From our experience, it would appear that younger and smaller animals are apt to show more frequently pale ears.

However, after considering all possible sources of error, the fact appeared to be unmistakable that subcutaneous injection of adrenalin often favors a primary dilatation of the blood-vessels of the rabbit's ears.

Investigators[3] who studied the effect of the suprarenal extract upon the general blood-pressure, have often made the assertion that sub-

[1] SCHIFF: Archiv für physiologische Heilkunde, 1854, xiii, p. 523.

[2] BECKE VAN DER CALLENFELS: Zeitschrift für rationelle Medizin, 1855, p. 157.

[3] GOTTLIEB: Archiv für experimentelle Pathologie, 1896, xxxviii, p. 99; LEWANDOWSKY: *Loc. cit.;* BORUTTAU: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 97.

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cutaneous injection induces no vasoconstriction. From our studies upon the blood-vessels of the rabbit's ear, we can positively state that a subcutaneous injection of a sufficient dose of adrenalin will cause a distinct constriction of the vessels. In a young rabbit, of a weight less than 1000 grams, an injection of 1 c.c. of pure, commercial adrenalin (1 : 1000) will cause within ten minutes a distinct blanching of both ears. Usually the animal then becomes exceedingly prostrated. In an animal of 1500 grams and more, a subcutaneous injection of about 1.5 to 2.0 c.c. of adrenalin will cause a gradual, slow but distinct narrowing of the blood-vessels, especially noticeable in the larger ones; the central artery may finally appear as a fine line, sometimes unevenly constricted, moniliform, but it never disappears entirely.

When 0.6 to 1.0 c.c. of pure adrenalin is injected subcutaneously into a medium-sized rabbit, in most instances it may be observed that after a few minutes the periods of vasodilatation become steadily longer, and those of constriction shorter, until about ten to twenty minutes after the injection, when the dilatation remains practically stationary for a period of from ten to thirty minutes. The central artery stands out clearly in its entire length, and many very fine vessels become visible. As a rule, however, the small vessels disappear again soon, especially after the injection of a large dose, while the central artery remains dilated for some time. The dilatation of the vessels in these cases is not as intense as after an excitement or a struggle of the animal; it is more comparable to the dilatations seen generally after section of the sympathetic, which as a rule are also not very intense.

When a smaller dose is injected, the effect is sometimes restricted to an increase in duration and a more frequent appearance of the phases of dilatation, but then an additional injection of 0.5 c.c. or more will bring out a permanent vasodilatation. In very rare cases a short period of constriction seemed to precede the vasodilatation. We should add that injections of saline or water do not favor vasodilatation.

The following are abbreviated protocols of some of the experiments.

*Experiment 85. March 24, 1903.* — 10 A. M. Small gray rabbit, about 1200 grams. Ears watched for about half an hour in a cool room. Ears very pale, occasionally vessels fill faintly but constrict again quickly. Injected 12 minims of pure adrenalin. In a few minutes the ear-vessels fill up very well and remain full, except for very few and short intervals of con-

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striction, for about half an hour, when the vessels become narrower again; but even after forty minutes the ear is not as pale as before injection.

March 25. Injected 15 minims of saline, no effect.

March 26. Injected saline, no effect. Half an hour later injected 12 minims of adrenalin, — very definite dilatation though not as marked as on the 24th.

*Experiment 96. April 5.* — Large black rabbit watched for half an hour in a cold room. Blood-vessels vary, dilate and constrict; at first average duration of constriction five to eight seconds; of dilatation ten to fifteen seconds. Later the constriction periods become longer.

Injected 25 minims of adrenalin. During the first ten minutes slight variation, but the ear did not become really pale again, and the constriction lasted only a second or two. After this period, the vessels remained stationary in dilatation for 15 minutes, then became narrower again. Thirty-five minutes later vessels still somewhat dilated.

The results of this series of experiments would seem to have an unfavorable practical bearing upon the subcutaneous application of adrenalin for hæmostatic purposes. As we have seen above, only such doses of adrenalin as are large enough to cause a distinct and dangerous general muscular paralysis of the animal, can induce by subcutaneous injection a constriction of the blood-vessels. Only such doses, therefore, come into consideration in the employment of adrenalin as a therapeutic measure. On the other hand, medium doses of this drug cause a vasodilatation; therefore, instead of contracting, the bleeding vessel would become dilated, and the bleeding would be increased.

The results of the following series of experiments will throw some light upon a mechanism by which the suprarenal extract might indeed be capable of controlling certain forms of hemorrhage.

## Effect of Subcutaneous Injection after Elimination of the Auricular Vasomotors on one Side.

In our previous paper in this Journal[1] referred to above, we have studied the effect of intravenous injection of adrenalin upon the ear-vessels of rabbits after section on one side of practically all nerves carrying vasomotors for the ear. We found that the constriction on the operated side lasted considerably longer than on the normal side.

[1] S. J. and Clara Meltzer: *Loc. cit.*

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In a new series of experiments we studied, under the same conditions, the effect of subcutaneous injections. The remarkable results which we uniformly obtained are chiefly as follows: In every adult rabbit in which, on one side, the sympathetic was cut, the ganglion removed, the third cervical nerve and its connections cut, or in which ganglion and cervical nerves together were eliminated, a medium dose of adrenalin, when injected subcutaneously, brought out in every case a constriction of the vessels on the operated side, while on the non-operated side the vessels became more or less distinctly dilated. The constriction is a gradual one; it begins a few minutes after the injection, progresses slowly, and sometimes does not attain its maximum for half an hour. The constriction is rarely so great as after an intravenous injection; but it may last for many hours,—in fact, the vessels sometimes do not assume their original width before the following day. The behavior of the blood-vessels of the ear on the unoperated side differs but little from that reported above, when adrenalin is injected subcutaneously into normal rabbits. We shall only add that, if all conditions are equal, the vessels of the normal ear in an operated animal generally show, as it seemed to us, a somewhat lesser tendency toward frequent dilatations than those of a normal animal. Hence the dilating effect of adrenalin was more easily recognizable and appeared to be more marked in the operated animals than in the normal ones. It was in these animals that we first noticed the distinctly dilating effect of the subcutaneous injections. The constricting effect upon the ear-vessels of the operated side is present in all cases, whether the dilatation of the vessels was due to the section of the sympathetic or to that of the cervical nerves. In fact, the constriction is marked even if, before the injection, there was no greater dilatation than the usual width in normal animals; which is indeed often the case a few days after the section of the sympathetic. It appeared, however, that in most cases when the cervical nerves alone were cut, even if the dilatation consequent upon this section was considerable, the constriction after the injection of adrenalin set in later and appeared less pronounced than in the cases in which the sympathetic was cut.

In a few cases in which either the sympathetic alone or the cervical nerves alone were cut, the injection brought out either a constriction in the centre and a dilatation in the periphery of the ear, or *vice versa*, *i.e.*, a dilatation in the centre and a constriction in the periphery, thus showing in one and the same ear the dilating effect

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upon vessels with central innervation and the constricting effect upon those which are deprived of a central control.

In small and young rabbits, and by the use of very large doses, a subcutaneous injection brought out a constriction even on the unoperated side. But even in these cases the constriction set in later and was markedly less than on the operated side.

In many experiments a leg was very tightly constricted and adrenalin injected peripherally to the ligature. When, some time later, the ligature was removed, both ears blanched within a very short time, even after the use of medium doses. But even in these cases the constriction in the unoperated ear set in later and was distinctly less marked than in the operated ear. The cause of the greater efficiency of the subcutaneous injections in these experiments with ligation of an extremity, we shall have a better opportunity to discuss in a future paper on the influence of adrenalin upon the pupil.

The following are a few abbreviated protocols of the last series of experiments.

*Experiment 53. Dec. 1, 1902.* — Large brown rabbit anæsthetized. Ganglion pulled out and third cervical nerve cut on the right side.

Dec. 4. Right ear-vessels full; left pale.

2.45 P.M. Injected 25 minims of adrenalin 1 : 1000 under the skin of the back.

2.50 P.M. Right ear paler; left variable.

3.10. Right ear pale; left full and stationary.

Dec. 18. Right ear pretty well dilated; left pale. Injected 15 minims as above. Left soon became full, and remained so for several minutes, then gradually became paler. Right ear continued to pale gradually.

Dec. 23, 6 P.M. Injected 10 minims. In a few minutes vessels of left ear dilated; right ear pale.

9. P.M. Both ears returned to their previous states.

Dec. 24. Right hind leg tied. Injected below ligature 20 minims. Ligature removed after five minutes. Right ear immediately becomes completely pale; left ear a little fuller than before injection.

*Experiment 54. Dec. 1, 1902.* Rabbit anæsthetized. Left cervical nerve cut.

Dec. 15. Left sympathetic and also right third cervical cut.

Dec. 16, 8.30 P.M. Injected 30 minims adrenalin 1 : 1000 under the skin of the neck. In 6 minutes both ears pale, left entire ear, right-central artery still visible. Animal thoroughly prostrated; found dead next morning.

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*Experiment 59. Dec. 11, 1902.* Large gray rabbit anæsthetized. Left third cervical nerve and connections and left sympathetic cut.

Dec. 17, 8.20 P. M. Left ear very full; right ear pale.

8.27. Injected under skin of neck 25 minims adrenalin.

8.29. Left ear paler; right fuller. Redness of right continues, while left continues to get paler.

8.45. Right ear still moderately full; left pale.

*Experiment 62. Dec. 16, 1902.* Large white rabbit anæsthetized. Right ganglion removed and third cervical nerve and its connections cut.

Dec. 17. Vessels of right ear fully dilated; left ear very pale. Injected under skin of neck 20 minims adrenalin. In two minutes vessels of right began to contract; in 14 minutes right ear quite pale; left somewhat full.

Dec. 23, 6 P. M. Hind leg tied with Esmarch bandage, injected distally 30 minims; toxic symptoms while bandage still on; bandage removed, both ears very pale.

9 P. M. Left ear pale; vessels of right dilated. Animal recovered, and condition of ears as before injection.

Dec. 24. Injected subcutaneously 15 minims; vessels of left ear became fuller; right ear pale.

Jan. 14, 1903, 5.30 P. M. Right leg tied very firmly; injected below ligature 20 minims. No effect noted while ligature on.

7 P. M. Ligature removed; right ear blanches immediately; left blanches a little later.

Both our series of experiments establish beyond a doubt that the subcutaneous injection of a medium dose of adrenalin causes a dilatation of the vessels of the ears when they are under the control of central vasomotors, but it induces a distinct constriction of the ear-vessels when the central innervation is essentially eliminated. These medium doses are, however, far in excess of those which give in intravenous injections a maximum constriction. Above a certain minimum the smaller the dose the more distinct the dilating effect of the subcutaneous injection upon the vessels of the normal ears.

These results will be better understood when we recall the hypothesis which we offered in our last paper[1] in explanation of the facts recorded there, which hypothesis was the starting point for the present series of experiments. We have there put forward the assumption that the suprarenal extract, when reaching the central nervous system through the circulation, stimulates there the vasoconstricting as well as the vasodilating mechanisms. But in conform-

[1] S. J. and CLARA MELTZER: *Loc. cit.*

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ity with the mode of action of all stimuli upon the central vasomotor mechanisms, stronger stimulation by adrenalin favors vasoconstriction, and weaker stimulation favors vasodilatation. A large dose of adrenalin represents a strong stimulus, and a small dose a weak one. If a large (efficient) dose is introduced into the circulation it at once favors constriction. When, however, later on, by oxidation and elimination (and neutralization ?), the original dose becomes considerably reduced, the reduced dose still stimulates both mechanisms, but it now favors dilatation; hence the rapid disappearance of the constriction and the appearance of a dilatation beyond the normal, as an after-effect. When, however, the central influences are eliminated, there is no longer a central dilating force present to overcome the stimulation of the peripheral constricting mechanisms, for which the small dose is a sufficient stimulus; hence the prolonged constriction of the vessels in the ear deprived of its vasomotors.[1] If we were right in our assumption, there was then a possibility of finding a small dose which would primarily cause dilatation. In the experiments with intravenous injections, we were not very successful in finding such doses. Our attention was then turned toward the method of subcutaneous injections in the expectation that in this method only very small doses at a time will be absorbed into the blood. We have seen the positive result.

Our explanation of the results obtained in the present series of experiments is then as follows: When even a comparatively large dose of adrenalin is injected subcutaneously, only a very small fraction of it is absorbed at a time into the blood; some of it is soon eliminated and thus put out of service. The amount of the substance which is actually present in the blood is very small. When the vasomotor nerves are intact, the small dose of adrenalin in the blood favors vasodilatation. The result is that either the dilatation only exactly neutralizes the (central and peripheral) constricting effect, and there is consequently no change in the appearance of the vessels; or it more or less overcompensates the constriction; the effect is then a more or less distinct dilatation of the vessels. However, if the central innervation is eliminated, the small dose within the blood stimulates the peripheral constricting mechanisms without any antagonistic influences of central origin; hence the constriction of the blood-vessels of the ear on the operated side.

[1] For further particulars of this hypothesis we refer to the above-mentioned communication.

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We wish to add a few remarks. Our experiments show in the first place that the suprarenal extract is very effective upon the blood-vessels even when injected subcutaneously. It has been generally assumed, as mentioned above, that subcutaneous injection of the suprarenal extract exerts no influence on the blood-pressure, and this was explained by the assumption that the extract of the suprarenal capsule becomes oxidized in the lymph spaces before it can reach the circulation.[1] Recently[2] some reports were published to the effect that when very large fatal doses were used, the blood-pressure became affected even by subcutaneous injection. This, however, might be interpreted by assuming that the surplus of oxygen in the lymph spaces was insufficient to destroy all the adrenalin. Our experiments, however, show unmistakably that even comparatively small doses of adrenalin are effective upon the state of the blood-vessels from the subcutaneous tissues, the effect being either a dilating or a constricting one, depending upon the presence or absence of central vasomotor influences. That the suprarenal extract does not become oxidized by the tissues is best shown by the experiments with the injection into the tightly ligated legs. Although the substance remained in intimate contact with the tissues for hours, there appeared to be not even the slightest impairment of the drug after the ligature was removed.

The following consideration is of general interest. If we have the constricting property of the extract in mind, our experiments have taught us the fact that subcutaneous injection of rather large doses has no effect on the ear-vessels when the central innervation is normal, but it exerts a considerable influence upon the vessels as soon as the central innervation is eliminated. Now our knowledge of the effects of all drugs, alkaloids, toxins, or metabolic products, is mostly derived from a study upon normal animals or organs. Are the effects the same when the organs are deprived of their normal innervation? As far as we know, this question has as yet hardly been seriously raised. Our experiments have demonstrated that the effect on pathological organs can be diametrically opposite to that on the normal ones!

Finally we wish to recur again to the use of adrenalin as a hæmostatic. Above, we raised the point that if a permissible dose of

[1] LEWANDOWSKY and BORUTTAU: *Loc. cit.*

[2] AMBERG: Archives internationales de pharmacodynamie et de thérapie, 1902, xi, p. 57.

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adrenalin, when injected subcutaneously, dilates the vessels, the use of adrenalin would obviously be somewhat harmful. Another objection has often been made of late against the use of such drugs in hemorrhages as cause a contraction of all the blood-vessels in the body, that the general rise of blood-pressure will favor the escape of blood from the diseased or injured vessel. The harm would be greater than the good done by a possible moderate local contraction of this vessel. In our experiments, however, we have seen that a subcutaneous injection of a permissible dose of adrenalin causes a dilatation of the vessels in normal parts, while vessels which are deprived of normal innervation become constricted. Now if a hemorrhage occurs in a diseased part, there is reason to assume that the vasomotor innervation of these parts is already essentially injured. In this case a subcutaneous injection of adrenalin could have an ideal hæmostatic effect: it would lower the blood-pressure in the normal parts about the lesion, which would divert the influx of blood from the bleeding point, while it would cause at the same time a direct constriction of the bleeding vessels.

## Summary.

Subcutaneous injection of suprarenal extract exerts a distinct effect upon the blood-vessels of the ear. In normal animals a large distinctly poisonous dose causes blanching of the ears; a medium dose causes a moderate but distinct dilatation of the blood-vessels. In rabbits in which the vasomotor nerves were cut on one side, a subcutaneous injection of a medium dose of adrenalin induced a distinct constriction of the vessels on the operated side and a dilatation on the unoperated side.

Apparently adrenalin is but very little oxidized in the subcutaneous tissues.

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# DIFFERENCES OF POTENTIAL BETWEEN BLOOD AND SERUM AND BETWEEN NORMAL AND LAKED BLOOD.

By G. N. STEWART.

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IT is well known that, in general, the contact of solutions of different electrolytes, or of solutions of the same electrolyte of different strengths, gives rise to electromotive force. This was first explained by Nernst as due to the unequal velocity of diffusion of the ions. With the view of throwing further light on the properties of the envelopes of cells as regards their permeability for electrolytes, I have endeavored to determine, by Poggendorff's compensation method, whether measurable differences of potential exist between defibrinated blood, or sediments rich in corpuscles suspended in the serum of the same blood, and the serum separated by centrifugalization or obtained from the clotted blood; and between defibrinated blood and blood laked in various ways. The idea in the first case was to see whether the presence of the corpuscles, in virtue of the unequal permeability of their envelopes for different ions, would cause any electrical difference, and in the second case to see whether the liberation of electrolytes and hæmoglobin from the corpuscles, and the alteration in the permeability of their envelopes would develop a difference of potential. I had intended also to study the differences of potential between standard solutions of electrolytes (*e.g.* 0.9 per cent NaCl solution or serum) and suspensions of blood-corpuscles in the same solutions, plus definite quantities of electrolytes known to penetrate the corpuscles, and to compare those differences with the differences of potential between the same liquids when the blood-corpuscles are absent. These latter observations, however, I have not yet been able to make.

*Method.* — The two liquids occupied the two limbs of a U tube, into which dipped a pair of unpolarizable electrodes ($ZnSO_4$ clay mixed with 0.9 per cent NaCl solution). The glass tubes of the electrodes were straight, but tapered at the immersed ends so as to enter the U tube easily and to



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| Combination. | E. M. F. in volts. | Remarks. |
|---|---|---|
| Blood, water | 0.0215 | Blood –, water +. Laking only at interface. |
| Blood, water | 0.0057 | Partially mixed up blood in one limb of tube, the other remaining full of unaltered blood. |
| Blood, water | 0.0028 | Mixed up still more. |
| Sediment of blood, serum | less than 0.0002 | |
| Sapotoxin-laked blood, control | less than 0.0002 | To 5 c.c. of defibrinated blood, 0.9 c.c. of a 2 per cent sapotoxin solution in 0.9 per cent NaCl solution was added, and then, to make sure of maximum laking, a little sapotoxin in substance. The "control" consisted of blood to which as much 0.9 per cent NaCl solution was added as was added of the sapotoxin solution to the laked blood. |
| Blood, 0.9 per cent NaCl solution | 0.0183 | Blood –, NaCl solution +. The defibrinated blood was drawn 48 hours before. |
| Blood, 0.9 per cent NaCl solution | 0.0065 | Same blood, but after standing 48 hours more. |
| Blood, sapotoxin-laked blood | less than 0.0002 | Same blood. Enough sapotoxin was added in substance to cause complete laking. |
| Blood, sapotoxin-laked blood | 0.0036 | 0.0575 gm. sapotoxin in substance was added to 20 c.c. of defibrinated blood drawn 24 hours previously. Laking is complete in 2 minutes at room temperature. This observation of E. M. F. was taken 31 minutes after mixture of the blood and sapotoxin. Defib. blood +, sapotoxin blood –. |
| Blood, sapotoxin-laked blood | 0.0009 | Later on. |
| Mixed somewhat | 0.0005 | For the defibrinated blood, $\lambda$ (50) = 39.73. For the sapotoxin blood, $\lambda$ (50) = 63.22. For the serum, $\lambda$ (50) = 85.93. For serum in 20 c.c. of which 0.068 grams sapotoxin was dissolved, $\lambda$ (50) = 85.08. |
| Blood, heat-laked blood | less than 0.0001 | For defibrinated blood, $\lambda$ (50) = 39.73. For heat-laked blood, $\lambda$ (50) = 37.84. |
| Sapotoxin-laked blood, serum | less than 0.0001 | Many Hb crystals in the sapotoxin blood. |
| Defibrinated blood, serum | less than 0.0001 | |
| Blood, sapotoxin blood | less than 0.0001 | |
| Blood, 0.9 per cent NaCl solution | 0.0002 | Blood –, NaCl solution +. |
| Water-laked blood, defibrinated blood | 0.0003 | Two volumes water were added to the blood. Water-laked blood –, defib. blood +, E. M. F. seemed to decrease after a few minutes. |
| Defib. blood, water | 0.0018 | Defibrinated blood +, water –. |
| Defibrinated blood, sapotoxin blood | 0.0001 | Sapotoxin blood +, defibrinated blood –. For the sapotoxin blood, $\lambda$ (50) = 63.22. For the defibrinated blood, $\lambda$ (50) = 41.09. |
| Mixed-up blood in U tube | .... | E. M. F. same as before. |
| Putrid blood, fresh blood | less than 0.0001 | The putrid blood was not completely laked. Putrid blood +, fresh blood –. |

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fit snugly. The clay plugs were flush with the narrow ends. Before each observation, the difference of potential between the electrodes was determined by immersing them in a U tube filled with 0.9 per cent NaCl solution. Usually it was very small (less than 0.0002 volt), and unless this was the case, the electrodes were set up again. When the same electrodes were to be used for successive observations on more than one combination, their tips were immersed in test-tubes filled with 0.9 per cent NaCl solution. The U tube for the measurements and the test-tubes for the washing of the electrodes were always slipped into position, while the electrodes were separately clamped, and remained undisturbed throughout the experiment. When scrupulous care is exercised in preparing the electrodes at the start, good results can be obtained in this way without the necessity of setting up fresh electrodes for each fresh combination, results which, for the present purpose at least, can hardly be surpassed by those yielded by the so-called normal mercury-calomel electrodes. The resistance of the compensating wire (represented by a plug rheostat) was never less than 10,000 ohms.

It will be observed that even after the action of such drastic hæmolytic agents as sapotoxin, in doses sufficient to produce marked liberation of electrolytes from the corpuscles, the differences of potential between the laked and unlaked blood are very small. No definite differences could, in general, be made out between defibrinated blood, or a blood sediment very rich in corpuscles, and the serum separated from it.

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# THE ACIDITY OF URINE.

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## The Total Acidity of Urine.

NOTWITHSTANDING the large number of investigations which have been published regarding the acidity of urine, no method has yet been found for determining this factor with even approximate accuracy. The reason for this condition is the fact that no successful attempt has been made to overcome the two chief difficulties which make direct titrations of urine unreliable: namely, first, the occurrence of calcium in the presence of monobasic phosphates, and, secondly, the presence of ammonium salts.

The older methods of directly titrating the acidity with tenth normal sodic hydrate in the presence of various indicators have therefore been given up as worthless by most investigators, and the more recent methods have been directed toward the determination of the monobasic phosphates on the assumption that to these is due practically all of the acidity of urine.

These later methods have, however, been shown to be inaccurate for the determination of the phosphates, and are, moreover, certainly open to the fatal objection that a considerable part of the acidity of urine (sometimes more than one-half) is due to organic acids.

Two years ago Nägeli[1] took up again the method of directly titrating urine with tenth normal alkali, using phenolphtalein as indicator, and attempted to prove that this gives fairly accurate results. But it can easily be shown that the two most important experiments with which Nägeli supports his conclusion give, if properly performed, results contradictory to those found by him.

The experiments referred to are the direct titration of monocalciumphosphate, and the titration of any acid in the presence of ammonium salts. That monocalciumphosphate cannot be titrated with phenolphtalein and sodic hydrate under the conditions present

[1] Nägeli: Zeitschrift für physiologische Chemie, 1900, xxx, p. 313.



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in urine is seen at once when neutral calcium chloride is added to a titrated monopotassium phosphate solution. The addition of the calcium salt will in every case materially increase the apparent acidity of the solution. Nägeli seems to have assumed that the amount of calcium present in urine is too small to produce any noticeable error in the titration of its acidity. In this he is mistaken. The error due to the calcium of urine, as will be shown below, may amount to more than ten per cent of the total acidity found.

Nägeli's experiments with ammonium salt solutions are even more unsatisfactory. The apparent acidity of neutral ammonium salt solutions in the presence of phenolphtalein is so pronounced that phenolphtalein cannot be used as indicator in the presence of even such limited amounts of ammonium salts as occur in normal urine. Seventy milligrams of ammonium chloride, for example, containing about 13 c.c. $\frac{n}{10}$ $NH_3$ (an amount by no means rare in 25 c.c. concentrated urine) require in 100 c.c. of distilled water about 3 c.c. tenth normal sodic hydrate to give a fairly distinct pink coloration.

Nägeli has, therefore, by no means shown the old objections raised against the direct titration methods to be invalid, and it is clear that, in order to make such direct titration of the acidity of urine possible, the difficulties mentioned must be overcome.

The disturbing effects of the calcium salts of urine can be completely removed by means of a reaction discovered by Liebig. Liebig found that both di- and tricalcium phosphate can be "dissolved" by means of potassium oxalate. That this reaction is admirably suited to the purpose is seen from the following: A disodicphosphate solution is, owing to dissociation distinctly alkaline to phenolphtalein. If to such a pink colored solution is added calcium chloride, the pink color disappears, calciumphosphate is precipitated, and the solution is strongly acid. Adding a few crystals of neutral potassium-oxalate and shaking rapidly restores the original pink color of the solution, and the first flaky phosphate precipitate is replaced by a finely granular one of calcium oxalate. This transformation is quantitative, as can be shown by first adding potassium oxalate, and then calcium chloride, to a titrated monopotassium phosphate solution, — notwithstanding the presence of the calcium it will now be found, on titrating, that the acidity of the phosphate solution has not changed.

The principle of the above method for counteracting the tendency of calcium to form basic phosphates, as has already been mentioned,

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was discovered by Liebig over twenty years ago. L. de Jager[1] in his paper on the determination of calcium and magnesium in urine has used sodium oxalate for the same purpose.

In addition to the calcium, there are present in urine certain smaller amounts of magnesium salts which are also generally supposed to interfere with the direct titration of phosphoric acid. This is, however, not the case. Pure magnesium salt solutions when added to a disodicphosphate solution produce no precipitate, and do not change the reaction of the solution.[2] The addition of potassium oxalate to urine before titrating the acidity is therefore amply sufficient to eliminate any error that would otherwise arise from the formation of earthy phosphates.

The presence of ammonium salts in urine, on the other hand, seemed at first to offer almost insurmountable difficulties. If the ammonium salt simply increased the acidity, one could find how much this increase would amount to for a given quantity of ammonia, and then after determining this constituent make a corresponding correction in the acidity of the urine. This would indeed largely correct the error. But another objectionable feature is the fact that the ammonium salts give such a slowly increasing color with phenolphtalein and tenth normal alkali, that the end-point of the titration becomes exceedingly uncertain.

These difficulties due to ammonium salts cannot both be removed quantitatively, to be sure, but they can be lessened to the point where the error becomes negligible, by means of the same reagent which was used above, — potassium oxalate.

A neutral ammonium chloride solution was prepared, 1 c.c. of which contained 3.8 c.c. $\frac{n}{10}$ $NH_3$. 2 c.c. of this solution + 23 c.c. distilled water + 1 drop $\frac{1}{2}$ per cent phenolphtalein solution required (at 24°) 1.2 c.c. $\frac{n}{10}$ NaOH to produce a fairly pronounced pink coloration.

The experiment was repeated as above, except that before titrating rather more powdered potassium oxalate was added than could be dissolved on shaking one minute. In this case 0.4 c.c. $\frac{n}{10}$ NaOH produced about the same depth of color as in the previous experiment.

The 11.4 c.c. $\frac{n}{10}$ $NH_3$ contained in each of the above experiments

[1] JAGER: Centralblatt für die medicinischen Wissenschaften, 1902, p. 641.

[2] JAGER also overlooked the fact that phosphates can be titrated in the presence of magnesium salts without the production of basic magnesium phosphates; this renders his method for determining the magnesium in urine worthless.

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represents fully as much ammonia as is found in 25 c.c. normal urine having a specific gravity of not over 1.023.

5 c.c. of the above ammonium chloride solution (containing 19 c.c. $\frac{n}{10}$ $NH_3$) required in 25 c.c. of water 2.7 c.c. $\frac{n}{10}$ NaOH to produce the same pink coloration as above.

After adding an excess of potassium oxalate and shaking for one minute, 0.6 c.c. $\frac{n}{10}$ NaOH sufficed to produce about the same end-point in the titration.

Since 19 c.c. $\frac{n}{10}$ $NH_3$ represents more ammonia than occurs in 25 c.c. of normal human urine, the result of the last experiment may be said to represent the upper limit of the error that can arise from the ammonium salts in normal urine on titrating the acidity of the latter in the presence of an excess of potassium oxalate.

On titrating an acid solution, the error due to the ammonium salts might be thought to be greater because of the dilution that occurs on adding the alkali. As a matter of fact, this increase is very small in the presence of the oxalate, and is moreover just about neutralized in urine by the tendency of the dibasic phosphates formed during the titration to dissociate and give a distinct pink coloration with phenolphtalein a little before quite all the primary phosphate has been converted into the secondary phosphate.

To illustrate:

20 c.c. standard monopotassium phosphate containing 100 mg. $P_2O_5$ with a theoretical acidity of 14.1 c.c. $\frac{n}{10}$ required, after adding 5 c.c of the above ammonium chloride solution, and 15 gm. powdered oxalate, 14.7 c.c. $\frac{n}{10}$ NaOH to produce a distinct end reaction.

For determining the total acidity of normal human urine, I therefore propose the following simple method:

With a pipette transfer 25 c.c. of urine into a small Erlenmeyer flask (capacity 200 c.c.). Add one, or at most, two drops one-half per cent phenolphtalein solution, and 15 to 20 grams powdered potassium oxalate. Shake about one minute, and titrate *at once* with tenth normal sodic hydrate until a faint yet distinct pink coloration is produced. The flask should be shaken during the titration, so as to keep the solution as strong as possible in oxalate.

The following determinations taken from a recent metabolism experiment may be cited in order to illustrate the values involved on titrating the acidity of urine with and without the use of potassium oxalate:

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| No of urine. | Spec. grav. | $P_2O_5$ in mg. in 25 cc. | $NH_3$ in c.c. of $\frac{n}{10}$ in 25 c.c. | Total acidity in c.c. $\frac{n}{10}$ in 25 c.c. | | |
|---|---|---|---|---|---|---|
| | | | | Without oxalate. | With only enough oxalate to prec. the Ca. | With excess of oxalate. |
| 1 | 1.0225 | 74.0 | 9.0 | 13.3 | 12.0 | 10.8 |
| 2 | 1.025 | 85.5 | 9.2 | 14.4 | .. | 12.9 |
| 3 | 1.020 | 65.5 | 7.1 | 12.5 | 10.8 | 10.4 |
| 4 | 1.029 | 109.0 | 13.2 | 22.8 | 21.0 | 19.3 |
| 5 | 1.0305 | 117.5 | 14.9 | 27.5 | .. | 22.0 |
| 6 | 1.0245 | 73.5 | 10.1 | 15.0 | 13.3 | 12.6 |

It will be noted that the above directions for titrating the acidity of urine have not been applied to pathological urines containing ex cessive amounts of ammonia, such, for example, as are found during and preceding diabetic coma.

My intention was to work out a somewhat different procedure for such urines, but after having made a preliminary test on a urine containing 38.2 c.c. $\frac{n}{10}$ $NH_3$ in 25 c.c., I came to the conclusion that the method described above gives with certain precautions results that do not vary enough from the true values to make a separate method necessary.

The test was made as follows: 25 c.c. urine shaken with about 20 gm. potassium oxalate was titrated as above, except that a second Erlenmeyer flask containing 25 c.c. of urine and an excess of oxalate was kept as a standard, and the titration was continued only until the titrated sample showed a very faint darkening when compared with the standard. The acidity thus titrated = **9.2 c.c. $\frac{n}{10}$.**

25 c.c. of the same urine were then transferred to a round-bottomed litre flask. 50 c.c. tenth normal sodic hydrate and 50 c.c. methyl alcohol were then added, and the ammonia was distilled off at 50° C. in a vacuum, according to the Boussingault-Shaffer method.[1] 3.82 c.c. $\frac{n}{10}$ $NH_3$ was found on titrating the distillate. The remaining alkaline liquid in the litre flask was cooled, and after adding 25 c.c. $\frac{n}{10}$ HCl and a little potassium oxalate, it was titrated in the presence of phe-

[1] SHAFFER: This journal, 1903, viii, p. 345.

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nolphtalein with tenth normal sodic hydrate. The titration required 22.2 c.c. $\frac{n}{10}$ NaOH.

In this experiment the acidity of the urine is therefore $50 + 22.2 - (38.2 + 25) = 9$ c.c. $\frac{n}{10}$.

## Mineral Acidity of Urine.

By the term mineral acidity is meant the excess of the combining equivalence of all the mineral acids of the urine above that of the known bases, sodium, potassium, calcium, magnesium, and ammonia. The only available method at present for determining this excess is to determine separately each of the mineral acids, and each of the bases, and then to subtract the sum of the combining power of the bases from the sum of the combining equivalence of the acids. It need scarcely be mentioned that to determine all these constituents with the necessary degree of accuracy is a task which few investigators have cared to undertake.[1]

The importance of making a large number of observations on normal and pathological urines in regard to their mineral acidity or mineral alkalinity is, however, recognized.[2]

My own purpose in attempting to determine the excess of mineral acidity or alkalinity, was to try to discover whether there is any abnormality in the metabolism of the insane, and whether the urines of the insane show any indication of the occurrence of any unknown organic bases or acids.

As a result of considerable experimentation, I have come to the conclusion that the method which I am about to describe, and which has been in use for some time in this laboratory gives the true excess

[1] After the writing of this paper was completed, I received the April number of Beiträge zur chemischen Physiologie und Pathologie, which contains an article by R. Höber on the acidity of urine viewed from the standpoint of the ion-theory. Such a theoretical discussion of the subject was purposely left out of the present paper, but it will be found, I think, that the method here described is based upon a correct application of the ion-theory as it affects the dissociation of acids, salts, and indicators. Höber's deduction that phenolphtalein must, of necessity, be the most suitable indicator for titrating the acidity of urine, is only partly correct. He forgets that phenolphtalein can ordinarily not be used in the presence of ammonium salts.

[2] See Bunge: Lehrbuch der physiologischen und pathologischen Chemie, 1898, 4th ed., p. 348; Herter and Wakeman: New York University bulletin of the medical sciences, 1901, i, p. 7; Herter: Journal of experimental medicine, 1901, v, p. 617; Fr. Soetbeer: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 96.

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of mineral acids or bases in urine with as great accuracy as can be obtained with the present state of knowledge concerning the composition of urine.

The principle of the method is simple and by no means new. It has been used for determining mineral acids in vinegar and for determining the hydrochloric acid in stomach juice, but as far as I know has never been applied to urine. The method is as follows:

0.3 to 0.6 gm. of pure, dry, granular potassium carbonate is accurately weighed into a platinum dish, and 25 c.c. of urine are measured into it. The resulting alkaline solution is evaporated on the sand bath or electric oven to dryness, and when perfectly dry, the contents of the dish are burned below red heat over a so-called radial burner, giving a flame wide enough to heat the entire bottom of the platinum dish. The burning should continue for about an hour after all visible ammoniacal fumes have ceased to come off. At the end of an hour the flame is removed, and 10 c.c. of hydrogen peroxide water is added. The dish is then covered with a watch glass, and gently warmed until the peroxide is decomposed. The watch glass is then taken off, and the sputterings rinsed into the dish by means of a little water. The contents of the dish are again evaporated to perfect dryness, and are then again heated over the radial burner as before for about an hour.

The burning residue is now dissolved in water with the help of an excess of tenth normal hydrochloric acid (75 or 100 c.c. $\frac{n}{10}$ HCl, depending on how much carbonate was taken), and is rinsed into an Erlenmeyer flask, boiled to drive off the carbonic acid, and cooled. The excess of acid is then titrated with tenth normal sodic hydrate in the presence of a small amount of potassium oxalate and two drops one-half per cent phenolphtalein solution.

Since the amounts of alkali and of acid added to the urine in the above procedure are known, the final titration gives the data for calculating the apparent excess of mineral acids or alkalies originally present in the urine. Before the final result is obtained, certain other factors must, however, be taken into account.

The following factors must be determined: (1) the alkaline strength of the potassium carbonate; (2) the acidity of the hydrogen peroxide; (3) the $SO_3$ content of the hydrogen peroxide; (4) the preformed ammonia in the urine; (5) the inorganic $SO_3$ of the urine, and, finally, (6) the total $SO_3$ found in the titrated solution of the urine residue.

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All these determinations are so easily executed as to require no description, and since both potassium carbonate and hydrogen peroxide can be kept unchanged for months in well-stoppered glass bottles, the first three determinations enumerated above need not be made more than once (for any given sample of carbonate and peroxide). The determination of the mineral acidity therefore requires: —

(1) The burning and titrating of the urine as described.

(2) One ammonia determination.

(3) Two sulphate determinations.

The calculation of the results is not complicated. The preformed ammonia, the acidity of the hydrogen peroxide, and the acidity due to the organic $SO_3$ of the urine, all in terms of tenth normal acid, must be subtracted from the apparent excess of acidity found on titrating the burned urine residue. The acidity (in c.c. of $\frac{n}{10}$) of the organic $SO_3$ is obtained by subtracting the sum of the $SO_3$ of the hydrogen peroxide and the inorganic $SO_3$ of the urine from the total $SO_3$ of the urine residue and dividing the amount so obtained in mg. by 8.

To illustrate:

> 25 c.c. of urine were burned with 0.5287 gm. potassium carbonate (7.76 mg. of which contained 1 c.c. $\frac{n}{10}$ alkali). The burned residue was boiled with 75 c.c. $\frac{n}{10}$ HCl, and the titration required 19 c.c. $\frac{n}{10}$ NaOH. An ammonia determination gave 5.2 c.c. $\frac{n}{10}$ $NH_3$ in 25 c.c. of urine. The total $SO_3 = 59.9$ mg.; the inorganic $SO_3 = 42.8$ mg. (10 c.c. of the hydrogen peroxide used contained 8.8. mg. $SO_3$ and 0.5 c.c. $\frac{n}{10}$ acid).

0.5287 gm. $K_2CO_3 = 68.1+$ c.c. $\frac{n}{10}$ NaOH } = 87.1+ c.c. $\frac{n}{10}$ NaOH.
NaOH added = 19 " " " }
HCl added = 75 c.c. HCl.

Apparent acidity of urine = 12.1 c.c. $\frac{n}{10}$ HCl.
Ammonia in 25 c.c. = 5.2 c.c. $\frac{n}{10}$ }
Acidity of $H_2O_2$ = 0.5 " " } = 6.7 c.c. $\frac{n}{10}$ HCl.
Acidity of organic $SO_3 = \frac{59.9-(42.8+8.8)}{8} =$ 1.0 " " }

Mineral acidity in 25 c.c. = 5.4 c.c. $\frac{n}{10}$ HCl.

A few explanations of the above somewhat condensed directions for determining the mineral acidity may be added.

1. The chief difficulty in burning urine residue completely free from organic compounds containing ammonia is one that is frequently overlooked, and the urine literature contains many instances in which this oversight has undoubtedly led to erroneous results. This diffi-

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culty is due to the urea and to a smaller extent to the alloxur bodies. Although urea is both unstable and volatile at higher temperatures, it is by no means an easy task to burn pure urea completely into carbonic acid and ammonia. When heated even to a red heat in a platinum crucible, it is converted into white stable cyanic and cyanuric acid derivatives which look like mineral matter. That this residue is actually figured as mineral matter in determinations of mineral residues of urine according to the methods given in textbooks is certain. In Neubauer and Vogel's "Analyse des Harns," for example (10th edition, 1898, page 703), one is particularly warned against heating too high, as otherwise abundant fumes of "alkali chlorides" are driven off. The alkali chlorides are, as a matter of fact, very little volatile, and the fumes warned against are nothing more or less than ammonium carbonate.[1]

On account of these difficulties encountered in attempting to drive off urea quantitatively by means of heat, it is necessary to heat a long time as is prescribed above, and to use a flame that will heat the entire bottom of the platinum dish at once. If only a Bunsen burner is used, the cyanogen compounds will melt and flow away from the hot part of the dish, and thus escape decomposition. The errors that arise from such incomplete decomposition of the nitrogen compounds may be so large as to make the determination worthless, nor can it be corrected by determining the remaining ammonia, since the cyanuric acids also have a strong acid reaction. Where there is any doubt as to whether the decomposition of nitrogenous compounds has been complete, the final filtrate should, however, be distilled with alkali and the ammonia determined, as this will indicate approximately the amount of the error.

2. The burning process described above does not always yield a perfectly white ash. The small amount of carbonaceous residue left behind can of course be filtered off, but this is not necessary, as it is never enough to obscure the end-point of the titration.

[1] The above statement does not mean that more intense ignition would give the correct amount of mineral residue. In my opinion it is, on the contrary, from the nature of the case, impossible to isolate the mineral constituents from human urine, or from the urine of carnivorous animals, by direct evaporation and ignition.

What has been said regarding the determination of the total mineral residue of urine is also true of potassium determinations, and it looks to me very doubtful whether more than a very small portion of the potassium determinations in urine recorded in the literature are even approximately correct on account of the contamination with ammonia.

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3. Pure, dry, granular potassium carbonate seems the best alkali to use when evaporating and burning the urine. It is much less hygroscopic than the ordinary powdered sodium carbonate, and can be kept for months in a small glass-stoppered bottle without changing its alkaline value. The alkaline hydrates of sodium and potassium are not suitable; first, because if standard solutions are added it takes much longer to evaporate to dryness, and, secondly, because the hydrates are much more volatile than the carbonates.

The potassium carbonate can be kept very conveniently in a "specific gravity" bottle. The amount of carbonate used for each determination should be, as is mentioned above, from 0.3 to 0.6 gm. Larger amounts can of course be taken if it should seem necessary, but numerous experiments have convinced me that nothing is gained by adding larger amounts, and to do so entails an enormously increased tendency to bumping, with consequent loss of alkali, during the evaporation.

4. The hydrogen peroxide is added to oxidize the thiocyanates and any small amounts of sulphide which may have been formed during the burning. Before deciding on hydrogen peroxide, several other oxidizing agents were tried, such as potassium chlorate, sodium peroxide, and oxygen gas; but hydrogen peroxide was finally chosen as being the most suitable. For the suggestion of using this reagent I am indebted to H. Wislicenus.[1] Wislicenus used perfectly pure hydrogen peroxide in his experiments, but this seems scarcely practicable on account of the difficulty of obtaining it. The commercial product is good enough. The necessary corrections for the $SO_3$ and the free sulphuric acid it contains are very easily made.

5. It may not be unnecessary to point out that in burning the urine residue as directed above in the presence of potassium carbonate, there is a certain danger that the gas may contain sulphur.[2] In my experiments this danger was avoided. The gas used is made from gasolene, and is free from any appreciable quantities of sulphur. This was tested by heating potassium carbonate alone for two hours, and then testing for sulphate. Since the flame used is very low there may not be much danger, and the sulphate determinations would soon indicate if any sulphur dioxide were absorbed from the gas; but where this is the case, an alcohol burner must be used.

[1] Wislicenus: Zeitschrift für analytische Chemie, 1901, xl, p. 441.

[2] See Carl Th. Mörner: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 311.

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6. A few words must still be added in explanation of the correction used for the organic sulphur of the urine, including the ethereal and the so-called neutral sulphur. I am well aware that it is arbitrary to figure exactly one half of this sulphur as inorganic acid, and the other half (of the chemical equivalence) as "organically" combined.

According to the present conceptions of the chemistry of the carbon compounds, all of the above sulphur equivalence is in fact "organic," because of the partial combination with organic radicles. Since sulphur is such a pronounced acid-forming element, and since the acidity of such compounds as the ethereal sulphates and sulphonic acids is so clearly due to the $SO_3$ group of such compounds, it would seem more logical in this connection to class the acidity derived from them with the mineral acidity.

Having accepted this classification, it still remains arbitrary to assume that the organic sulphur of urine actually is there as monobasic acids. In such compounds as the alkyl sulphides found in dog's urine, we know in fact that we are dealing with neutral and not with acid compounds.

The term "neutral sulphur," as used at present, is however, in my opinion, a misnomer, since we do not know that more than an exceedingly minute fraction of the organic sulphur in human urine is there in neutral form. Ethereal sulphates, chondroitin-sulphuric acid, and the sulphocyanides we know, on the other hand, to be monobasic acids, and it seems quite as probable that most of the other organic sulphur compounds existing in urine will be found to have the sulphur with a free monovalent acid equivalence as to have it "neutral." Small traces of sulphur occurring in the mucoid and similar substances may, on the other hand, be spoken of as neutral.[1] The error involved in assuming that the organic sulphur of the urine figures as monobasic acids is, therefore, it seems to me, in all probability so small as to be negligible.[2]

[1] Urines containing appreciable quantities of albumen must be heated to boiling after acidifying with *pure* acetic acid and the clear filtrate taken for the mineral acidity determination. Very small quantities of albumen do not contain enough sulphur to interfere to any noticeable extent with the mineral acidity determination.

[2] In order to avoid some criticism perhaps the "organic phosphates" should also be mentioned. These have not been overlooked, but the determinations recorded in the literature, as well as a number of determinations made in this laboratory by Mr. Philip Shaffer, indicate that the organic phosphates are present, if at all, in such minute quantities as to have no determinable effect on the acidity of urine.

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The following experiments are added in order to indicate how the accuracy of the method was established.

1. 0.4560 gm. potassium carbonate was weighed into a large platinum crucible weighing 39.8760 gm. 0.895 gm. perfectly pure urea was added, and the crucible was then heated on the electric oven till no ammoniacal fumes were visible. The crucible was then heated over a radial burner one hour. After adding and evaporating 5 c.c. of water, the crucible was again heated to just below redness for one hour.

   At the end of this time the crucible and contents weighed 40.2797 gm. The crucible was heated for another hour, and now weighed 40.2798 gm. 40.2797 — 39.8760 = **0.4037** gm. (the weight of the carbonate made absolutely anhydrous). The theoretical weight of 0.4560 gm. of the potassium carbonate (7.76 mg. of which = 1 c.c. $\frac{n}{10}$ HCl) was **0.4054** gm.

   The heated carbonate was then dissolved in water and boiled with 75 c.c. $\frac{n}{10}$ HCl. After cooling, the titration required 16.5 c.c. $\frac{n}{10}$ NaOH. The value of the carbonate remaining was, therefore, 75—16.5 c.c. = **58.5 c.c. $\frac{n}{10}$ NaOH.**

   Theoretical = **58.76 c.c. $\frac{n}{10}$ NaOH.**

2. 0.3796 gm. potassium carbonate (corresponding to **48.93** c.c. $\frac{n}{10}$ NaOH) was weighed into a platinum dish. About 1 gm. of Kahlbaum's pure lactic acid (sp. gr. 1.21) and some water were added. The mixture was evaporated and burned two hours. In order to burn the charred residue more completely, small quantities of distilled water were twice added and evaporated, and the burning continued for fifteen minutes after each evaporation.

   The residue was then dissolved in water and boiled with 60 c.c $\frac{n}{10}$ HCl. The titration required 11.2 c.c. $\frac{n}{10}$ NaOH.

   The value of the carbonate recovered was therefore 60 — 11.2 = **48.8 c.c. $\frac{n}{10}$ NaOH.**

3. 25 c.c. urine burned with 0.6096 gm. potassium carbonate gave a mineral acidity of 3.4 c.c. $\frac{n}{10}$ in 25 c.c.

   50 c.c. of the same urine burned with 0.5513 gm. potassium carbonate gave a mineral acidity of 7.05 c.c. $\frac{n}{10}$ in 50 c.c.

## Organic Acidity in Urine.

Having described in the preceding pages how to titrate the total acidity of urine, and how to determine the mineral acidity of the same, but little need to be said in regard to the determination of the organic acids of the urine.

By subtracting the mineral acidity from the total acidity is obtained the organic acidity, or rather the total equivalence of organic

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acid, whether free or combined. This deduction is true even for pathological urines containing excessive amounts of combined organic acids such as are found in diabetic coma, only in these cases

| Urine No. | Specific gravity. | Total acidity. | Mineral acidity. | Organic acidity. |
|---|---|---|---|---|
| SERIES No. 1. | | | | |
| 1 | 1.025 | 12.9 | 4.7 | 8.2 |
| 2 | 1.020 | 10.4 | 3.1 | 7.3 |
| 3 | 1.029 | 19.3 | 8.4 | 10.9 |
| 4 | 1.0305 | 22.0 | 4.9 | 17.1 |
| 5 | 1.0245 | 12.6 | 4.4 | 8.2 |
| SERIES No. 2. | | | | |
| 1 | 1.0175 | 8.0 | 3.2 | 4.8 |
| 2 | 1.0185 | 10.0 | 5.4 | 4.6 |
| 3 | 1.018 | 9.0 | 3.2 | 5.8 |
| 4 | 1.027 | 15.0 | 8.5 | 6.5 |
| 5 | 1.027 | 16.4 | 7.9 | 8.5 |
| 6 | 1.0175 | 7.0 | 2.0 | 5.0 |
| SERIES No. 3. | | | | |
| 1 | 1.021 | 9.8 | −3.4 | 13.2 |
| 2 | 1.021 | 11.0 | −4.3 | 15.3 |
| 3 | 1.020 | 9.0 | −3.8 | 12.8 |
| 4 | 1.022 | 9.0 | 0.4 | 8.6 |
| 5 | 1.0225 | 9.5 | 0 | 9.5 |
| 6 | 1.020 | 9.0 | −1.5 | 10.5 |

the mineral acidity is algebraically negative, *i. e.* is replaced by mineral alkalinity, and the original total acidity may be said to be due wholly to organic acids. It is also true for urines which are directly

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alkaline to phenolphtalein, as becomes evident by expressing the original alkalinity in terms of algebraically negative acidity.

To illustrate: A mixture of sodic acetate and acetic acid would have an original acidity corresponding to the amount of uncombined acetic acid. The total amount of organic acid in such a mixture would be obtained by making a mineral alkalinity determination and adding the result so obtained to that obtained by the direct titration.

It has been very generally assumed that the acidity of urine is almost wholly due to the presence of monobasic phosphate, and that the organic acids of normal urine play but a very subordinate rôle. The extent to which the actual acid H ions of urine are derived from phosphoric acid depends on the amounts of the organic acids present, since these are in general much the weaker acids and therefore combine to a correspondingly smaller extent with the bases present.

It is practically impossible to determine the exact point of equilibrium in such a complex unknown mixture of acids as is contained in urine, nor is this point of any very great importance. For practical purposes it is, therefore, far nearer the truth to assume, as is done in this paper, that the organic acids are free, and that the excess acidity only is due to "acid phosphates." If this is done, it will be found that the acidity due to the phosphates varies within very wide limits, but that the greater part of the acidity of urine is in most cases due to organic acids. This is illustrated by the following acidity determinations taken from some recent metabolism experiments. All these acidity determinations were made in 25 c.c. of urine.

It will be noticed that the urines in the last series (No. 3) contained no mineral acidity, but on the contrary a rather pronounced mineral alkalinity. These urines were highly unusual in that 20 per cent or more of the total nitrogen was present as ammonia. Notwithstanding this extraordinary high percentage of ammonia nitrogen, the acidity determinations proved at once that oxybutyric or other organic acids were not present to any extent that could be considered pathological.

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# A STUDY OF THE REACTIONS AND REACTION TIME OF THE MEDUSA GONIONEMA MURBACHII TO PHOTIC STIMULI.[1]

By ROBERT M. YERKES,
WITH THE ASSISTANCE OF JAMES B. AYER, JR.

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## PROBLEMS.

THE experimental study which is the subject of this paper[2] is a continuation of work begun at Woods Hole three years ago. Certain of the earlier results have already been published in this journal.[3] In my first paper, which dealt with the sensory reactions of Gonionema, some of the organism's reactions to light were briefly described, and attention was called to their probable significance in the life of the medusa.[4]

[1] The experimental work of this paper was done at Woods Hole during the summer of 1902. I desire to express my thanks for the assistance rendered me by the authorities of the Marine Biological Laboratory.

[2] This paper is one of a series of Comparative Reaction-Time Studies, of which one dealing with the reaction time of the frog has already been published in the Harvard Psychological Studies, 1903, i, pp. 579–638; Psychological Review monograph, iv.

[3] YERKES, R. M.: This journal, 1902, vi, pp. 434–439. *Ibid.*, vii, pp. 181–198.

[4] YERKES, R. M.: This journal, 1902, vi, pp. 445–447.



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In the present work attempts are made (1) to note and analyze the various forms of reaction to photic stimuli exhibited, (2) to discover their relations to the life-history, habits, and instincts of the organism, and (3) to study the time relations of the various reactions in the hope of discovering something concerning the rôle of the nervous system. Although this paper is chiefly a discussion of the influences of certain environmental factors on reaction time, the central problems of the whole of the investigation are those of the functional importance of the nervous system.

## Reactions to Light and Gravity.

In its natural habitat, attached to marine plants or resting on the bottom at a depth of several feet from the surface of the water, Gonionema is exposed to intensities of light which are usually less than that of daylight. When it is in a dense mass of eel grass, as is usually the case at Woods Hole, the light is very weak, but when it happens to come to the surface during the day it is exposed to light which may be of the intensity of direct sunlight. When the weeds to which Gonionemata are attached are disturbed the animals are either torn from their attachments, or are stimulated by the disturbance of the water to release their holds, and immediately swim upward. If the distance to the surface of the water is not more than three to four feet, they usually continue swimming until the apex of the bell reaches the surface; they then turn over quickly. As soon as the medusa has turned the apex of the bell downward, it ceases contracting and passively sinks in the water; this continues until it comes in contact with something to which it can attach itself by the tentacles or manubrium. Animals which start upward at a great distance from the surface become fatigued before they appear at the surface, and sink back; but usually they do not turn over as do those which reach the surface. It is noticeable that at low tide many more small individuals come to the surface after the water has been stirred than at high tide. Since the number of large animals does not vary markedly with the tide, it would appear that the small ones are not able to swim to the surface when the distance is relatively great.

Why do the animals swim surfaceward instead of in the opposite direction when they leave their attachment? Evidently there are at least three factors which might determine the direction of movement either separately or working together: gravity, pressure, and light.

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When a Gonionema swims upward it is swimming against gravity, — that is, it is negatively geotactic. The medusæ show the same kind of reaction when they swim against a stream of water. While reacting positively to the tendency of gravity the animal is moving into regions of lower pressure, inasmuch as this force varies directly with the depth of water, and it is also coming into more intense light. Which, if any, of these agencies determines the direction of the animal's movement?

That light is not the all important cause can be shown by placing the medusæ in a vessel which is illuminated from below alone. Under such conditions they still swim upward when disturbed. That pressure is not of prime importance is proved by putting the animals into solutions of different densities, as well as by the use of sea-water with layers of other fluids above it. The animals uniformly move upward. It is probable, then, that gravity is chiefly responsible for the reaction. If a small piece of cardboard or wood is placed on the surface of the water, animals coming against it continue swimming just as they do when free in the water. Often they continue until exhausted, when they sink, not by turning over, but as do individuals which have failed to reach the surface. Although negative geotaxis seems to be the most important feature of this reaction of Gonionema, it is not probable that light is without influence; it is simply not the prime and necessary factor in the determination of the reaction.

In the reactions thus far described, we cannot say that Gonionema is positively phototactic, for although it does move toward the source of light, and into greater intensities, when swimming surfaceward, light is not the cause of the movement. On the other hand, experiments in the laboratory have shown that the animals are positively phototactic to certain intensities of light.[1]

## Reactions to Sunlight.

In this connection reactions to direct sunlight are to be considered. When Gonionemata in a glazed white earthenware dish are placed in sunlight, they at once become exceedingly active. At first they swim surfaceward, often forcing the apex of the bell several millimetres out of the water as they reach the surface; later many cease coming to the surface, while others begin to swim downward, bumping against the bottom of the dish; after a still longer interval of exposure, some

[1] Yerkes, R. M.: This journal, 1902, vi, p. 446.

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are found to move upward with the contractile phase of each beat and downward with the expansile phase. If now the sunlight is cut off from one end of the dish, thus leaving one portion bright and the other shaded, in a few minutes most of the animals will be in the shaded portion.

Careful observation reveals the following reaction as an explanation of this fact. When an individual in swimming about chances to cross from the sunlit region into the shadow, it very quickly ceases swimming and sinks to the bottom. If, later, in swimming about it chances to cross from the shaded region into the sunlight, it in most cases immediately ceases swimming, turns over, and sinks passively to the bottom. But, in this case, when it again becomes active, it does not move indifferently in any direction, as it does when in the shadow; instead, it usually turns in such a way as to move back into the shaded region. Because of this reaction, the animals rapidly gather in the shadow. Both *the sudden decrease in light intensity*, as the animal passes from sunlight to shadow, *and the sudden increase*, as it passes in the opposite direction, *temporarily inhibit activity*.

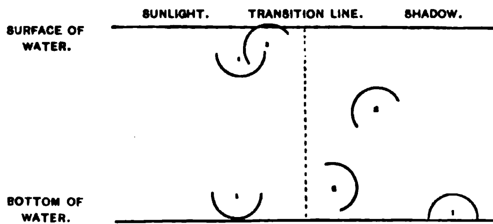


Diagram indicating the various positions assumed by a Gonionema in the sunlight-shadow reaction.

To indicate the usual number of animals in the sunlight and in the shadow, and also the number of times that animals react to the sunlight by sinking and then swimming back into the shadow, the following series of observations is given. During a period of forty-five minutes (see Table I), fifty-four reactions to the transition line, as the region of change of light intensity may be termed, were observed; of this number forty were of the type already described, *i.e.*, the animal ceased swimming as soon as it entered the sunlight, turned over, sank to the bottom, then, after a short interval, swam back into the shadow. The remaining fourteen cases were those of continued swimming in the sunlight. In almost three cases in four the medusæ swam back into the shadow instead of continuing their activity in the sunlit portion of the dish, as they would evidently have done had the intensity of illumination been the same throughout the dish.

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TABLE I.

SHOWING THE NUMBER OF GONIONEMATA IN SHADOW AND IN SUNLIGHT.

| Time. | No. in shadow ($\frac{1}{4}$ of dish). | No. in sunlight ($\frac{3}{4}$ of dish). |
|---|---|---|
| a. m. 10.00 | 8 | 4 |
| 10.05 | 9 | 3 |
| 10.10 | 8 | 4 |
| 10.15 | 11 | 1 |
| 10.20 | 10 | 2 |
| 10.25 | 9 | 3 |
| 10.30 | 11 | 1 |
| 10.35 | 10 | 2 |
| 10.40 | 11 | 1 |
| 10.45 | 12 | 0 |
| Total . . . | 99 | 21 |

The reaction just described is to all appearances a perfectly definite response to sunlight. But, one may ask, is it not a response to heat instead of to light? To obtain evidence on this point a two-inch screen of alum solution was placed between the sun and the vessel in which the medusæ were swimming. This screen cut off the heat, whilst it permitted the light to pass with diminished intensity. Under such conditions the animals reacted in essentially the same way. We may therefore conclude that sunlight, by virtue of its intensity, causes a negatively phototactic reaction in Gonionema.

During experiments with sunlight it was discovered that the medusæ after being exposed to the light for a short time begin to try to avoid it. When first exposed, they swim upward as in their natural habitat, whereas after an interval, dependent upon the intensity of the light and the condition of the animals, they swim downward and persistently bump against the bottom or edges of the vessel. If a number of Gonionemata differing in size, sex, and sexual condition, are exposed to the sunlight as described, those with mature sexual products (heavy gonads) are the first to try to avoid the light, and later the others react in similar fashion. Clearly the animals are

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attuned, so to speak, to a certain range of light intensity, and are negative in their reactions to higher intensities. The "sexually ripe" individuals are more sensitive to sunlight, and react more violently to it than immature individuals. The animals discharge their sexual products when in very weak light or darkness.

## The Directive Influence of Light.

Previously statements have been made concerning the directive influence of stimuli.[1] Weak light stimulates the animals to activity, but apparently does not influence the direction of their movements to any marked extent; light of medium intensity seems to cause movement toward the source of light at first, but it is later avoided; finally, strong light, although it may attract at first, soon repels the animals. The greater the intensity, the greater the directive influence of light. We must now inquire, how does light direct the movements of Gonionema?

This question can be answered satisfactorily by certain simple experiments. In the first place we have seen that Gonionema when it sinks to the bottom after coming into sunlight usually turns back toward the dark. This apparently is accomplished by the more forceful and earlier contraction of that side of the bell furthest away from the shadow. That this inequality of contraction is due to difference in stimulation seems probable, since the side away from the shadow is likely to be exposed to slightly more intense light than the remainder of the bell.

Clearer and more satisfactory evidence of the directive value of stimuli in support of this statement is furnished by experiments with tactual and electrical stimuli. When a Gonionema, resting on the bottom of a dish with the apex of the bell up, is touched at some point on the margin, *it reacts by swimming upward and away from the side stimulated* (Fig. 1). In not more than ten per cent of the cases does an animal thus stimulated swim toward the region of stimulation. If the animal chances to be resting bell down, the same direction of movement is taken, but the position of the animal necessitates such a turning as Fig. 2 represents. Fig. 1 shows the direction of movement when the stimulus is applied at *S* to an individual resting bell up. This reaction is much more satisfactory and definite when electrical stimulation is employed, since it does not involve the inter-

[1] Yerkes: This journal, 1902, vi, p. 447.

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ference with the organism's movement which touching it is likely to cause. There can be no doubt that the direction in which the bell turns is determined by inequality of contraction. The region stimulated contracts first and most strongly, and the water thus being driven out of that side of the bell tends to force the bell forward on the stimulated side, therefore turning the region stimulated upward if the animal is bell up, and downward if it is bell down. Any one who tries the experiment of approaching electrodes to a point at the margin of Gonionema will get a beautifully definite demonstration of the directive influence of a localized stimulus.

FIG. 1.—Direction of movement when animal is stimulated in "bell-up" position. *S* marks the region stimulated.

FIG. 2.—Direction of movement when animal is stimulated in "bell-down" position. 1 marks the position when stimulus was applied; 2 indicates the direction of movement.

In sunlight the medusæ at first come so forcibly to the surface that half the bell may appear above the water. Usually a bell thus forced out of the water inclines slightly to one side, and thus tends to turn the animal over, so that the observer can never be quite sure whether the turning is due altogether to the action of gravity, or whether it is in part due to some peculiarity in the final contraction of the bell. After having been in sunlight for a few minutes, the animals come to the surface with noticeably less impetus, and soon they fail to reach the surface at all. But even in the latter case they turn over, just as they do in ordinary light after they reach the surface. Under these conditions the turning due to stimulation by light evidently is accomplished by the bell contraction. Observation indicates that the side of the organism which is exposed to the most intense light contracts first and most strongly, thus forcing the bell over. As soon as the bell is downward, movement ceases and the animal sinks passively.

Thus far we have learned that Gonionema is either positively or negatively phototactic, according to the intensity of the stimulus, that its activity, *under certain conditions*, is inhibited by strong light, that light and not the heat accompanying it is responsible for the reactions described, and that the direction of its movements is definitely determined by light as well as by other localized or unequally applied stimuli.

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## Effects of Increase (Light) and Decrease (Darkness) of Photic Stimulation.

We now come to the problem of inhibition of activity which has appeared in various forms during the experiments. Berger[1] thus summarizes the results of a study of Charybdea made by him and Conant: "We have seen that it is very sensitive to light, strong light as also darkness inhibiting pulsations, while moderate light stimulated it to activity. Also, a sudden change from weaker to stronger light, or *vice versa*, may inhibit or stimulate to activity respectively. This behavior of Charybdea seems to be correlated with its habit of life on the bottom. We have no reason to doubt but that the eyes of the sensory clubs are the seat of light sensation." And Romanes,[2] who has studied the reactions of many kinds of jelly-fishes, remarks that Sarsia is sensitive to light, and usually responds to a flash by one or more contractions of the bell. He, moreover, believes that the change from light to darkness is inhibitory of activity.[3]

In studying the reactions of Gonionema to photic stimuli or the "photoskioptischen Sinn" (light-darkness sense), as Nagel[4] has named this kind of sensitiveness, I have found that Berger's descriptions of the reactions of Charybdea do not hold true for Gonionema, and further, that his statement that Gonionema's activity is inhibited by strong light is misleading.

Gonionemata when taken from the sea and placed in ordinary daylight are active for a long time. Whenever the intensity of the light is considerably decreased, they come to rest on the bottom of the vessel. Any sudden increase in the light causes them to become active again. In general, we may say that increasing the intensity of light causes Gonionema when at rest to react by contracting the bell; decreasing the intensity causes no reaction uniformly, although in exceptional cases an animal may react regularly to decrease in

[1] Berger, F. W.: Memoirs of the Biological Laboratory of Johns Hopkins University, 1900, iv, p. 22.

[2] Romanes, G. J.: Jelly-Fish, Star-Fish, and Sea-Urchins; being a research on Primitive Nervous Systems. New York, 1885, p. 39 *et seq.*

[3] For discussion of the stimulating effect of decrease in illumination, see Rawitz, B.: Jenaische Zeitschrift, 1892, xxvii, pp. 1-232; Drost, K.: Morphologisches Jahrbuch, 1887, xii, pp. 163-202; Nagel, W. A.: Der Lichtsinn augenloser Thiere Jena, 1896.

[4] Nagel, W. A.: Biologisches Centralblatt, 1894, xiv, pp. 385-390.

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intensity, and fail to react to increase. If, however, a swimming Gonionema is stimulated by an increase in the intensity of light it expands and settles to the bottom. So, likewise, an individual resting quietly in strong light may very frequently be made to react by cutting off the light. Increase in light intensity uniformly causes a visible reaction in from one to thirty seconds; but the nature of the reaction depends upon the condition of the animal at the instant of stimulation. If it is in a state of expansion (inactive), the light causes it to contract, hence the locomotor reaction; if it is in a state of contraction (active or inactive), the light causes it to expand, hence the absence of a locomotor reaction in the presence of a visible motor reaction. Decrease in light intensity almost uniformly causes a contracted animal to expand and come to rest, but it does not, except in certain unusual cases, cause an individual which is resting in a state of expansion to contract and become active. Excised margins quite commonly react to decrease as well as to increase of light intensity by contracting, but seldom are normal individuals found which show this uniformity.

Berger's statement would be strictly correct if he had said that the influence of strong light on an active Gonionema is to inhibit its activity. In view of the above facts, it is clear that increase in the intensity of light has in part the same effect upon an active Gonionema as has decrease in intensity; both tend to inhibit activity. On the other hand, a quiescent animal is stimulated to activity by increase in the light intensity, while it gives no visible reaction uniformly to decrease in intensity. Obviously we cannot say, then, that strong light and darkness always inhibit activity. There can be no doubt that in the majority of cases light, *i. e.*, increase in the intensity of illumination to which an animal is exposed, causes activity, whereas darkness tends to keep the animal at rest. In the study of reaction time to photic stimuli to be considered later, the quieting influence of darkness and the stimulating influence of light were made use of as experimenting conditions. The animals when covered, in a dish, soon come to rest, and when uncovered and thus exposed to more intense light react quickly by contracting the bell.

The reaction of Gonionema to sunlight which has been described is now seen to be in agreement with the results obtained with daylight.[1] In the experiments with sunlight the animals emerged from a weaker into a stronger intensity of light; hence, their activity was

[1] YERKES: This journal, 1902, vi, p. 446.

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temporarily inhibited, and they sank to the bottom. In a few minutes, however, the strong light forced a motor reaction which for reasons already pointed out resulted in a movement toward the shadow.

What may be conceived as the relation to the habits of the medusa of this temporary inhibitory effect of increase in light intensity is of interest. Gonionema when it reaches the surface of the water, after being disturbed in its habitat, turns over, and by reason of the influence of the light, or if not because of it, at least in the presence of this factor, passively sinks downward with fully expanded bell and tentacles. As it slowly sinks in this condition, it has a far better chance of coming in contact with food than it would if it swam down rapidly with contracted tentacles. A similar line of reasoning is applicable when we consider the position which is assumed by the sinking animal. It generally sinks with the bell pointed downward and the manubrium swinging free in the water above the bell. Now, as the animal sinks, any prey which becomes ensnared in the tentacles can readily be carried to the lips by the movement of the tentacles upward with the current, whereas, since the animal is sinking, it would be difficult for the organs to carry food downward against the water pressure and enable the lips to seize it. It may be maintained, therefore, that the reaction is especially suitable for the obtaining of food.

Light, having been one of the most important stimuli for the initiating of the surface reaction, — *i. e.*, turning, inhibition of activity, expansion of organs, — may finally have come to be the cause of a very similar reaction in the presence of other conditions, so that increase in light intensity now uniformly causes an active Gonionema to expand fully for a few seconds and permit itself to sink passively.

## The Reaction Time of Gonionema to Photic Stimuli.

**Method.** — Since a resting Gonionema almost invariably responds to an increase in light intensity with a sharp locomotor contraction of the bell, and since the time of this reaction is of such length as to be measurable with considerable accuracy by means of a stop watch, the light reaction of this organism offers an excellent opportunity for the study of the influence of various environmental conditions on the time of reaction.

The remainder of this paper is a description of experiments made to determine: the influence of intensity of light on reaction time;

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the influence of temperature on the reaction time to light; and the relation of sex, sexual condition, size of organism, and pigmentation to reaction time.

The following simple method served in the study of reaction time to light. Into a white earthenware dish containing about two inches of sea-water, placed before a window, a single Gonionema was put. So long as the animal was exposed to the ordinary daylight, it was active much of the time; but by placing a piece of card-board over

TABLE II.

REPRESENTATIVE SERIES OF REACTION TIMES TO DAYLIGHT.

| Experiment. | Animal No. 3. 14 mm. in diameter. | Animal No. 4. 21 mm. in diameter. |
|---|---|---|
| | secs. | secs. |
| 1 | 9.0 | 3.5 |
| 2 | 7.2 | 5.0 |
| 3 | 6.3 | 7.7 |
| 4 | 5.9 | 9.6 |
| 5 | 8.1 | 7.7 |
| 6 | 11.2 | 8.3 |
| 7 | 1.5 | 12.2 |
| 8 | 8.0 | 5.1 |
| 9 | 5.8 | 4.7 |
| 10 | 5.1 | 5.4 |
| Average | 6.8 | 6.9 |
| Mean variation | 1.89 | 2.18 |

the dish to weaken the light considerably, the animal could be brought to rest in a short time. Trial showed that from one to five minutes was sufficient to allow the animal to settle down. If after a certain period the dish was quickly uncovered the medusa reacted to the increase in light by sharply contracting the bell. The time of this locomotor reaction was determined thus. The instant the experimenter removed the cover from the dish he started a stop watch, and the instant the animal reacted he stopped the watch and noted the time of reaction. Then, after permitting the animal to

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swim about actively in the daylight for a period equal to that during which the dish had been covered, he again covered the dish. In the following experiments, unless otherwise indicated, the time of exposure to both intensities of light was five minutes, that is, the dish was covered for that period, and then uncovered for the same period.

TABLE III.

REACTION TIMES TO THREE INTENSITIES OF LIGHT.

| Animal. | Weak light. | | Medium light. | | Strong light. | |
|---|---|---|---|---|---|---|
| | Mean. | Mean variation. | Mean. | Mean variation. | Mean. | Mean variation. |
| 1 | 8.8 | 1.62 | 6.0 | 1.58 | 4.3 | 1.09 |
| 2 | 11.0 | 4.67 | 10.3 | 7.76 | 4.5 | 1.16 |
| 3 | 13.5 | 9.16 | 6.9 | 3.95 | 24.0 | 12.92 |
| 4 | 29.8 | 20.34 | 4.3 | 0.74 | 15.2 | 7.16 |
| 5 | 7.2 | 3.21 | 4.6 | 1.26 | 8.7 | 3.08 |
| 6 | 6.7 | 1.86 | 4.9 | 1.09 | 6.7 | 2.03 |
| 7 | 11.2 | 3.67 | 8.7 | 3.49 | 6.5 | 1.93 |
| 8 | 8.8 | 2.63 | 11.0 | 6.42 | 4.7 | 0.87 |
| General av. | 12.1 | 5.89 | 7.1 | 3.29 | 9.3 | 3.78 |

Generally ten reactions of each animal were measured. Table II gives two representative series of reaction times. The time of reaction to daylight is ordinarily about seven seconds, and as can be seen from the table, the variability is about two seconds. In no case was a slow contraction of the bell which did not introduce a locomotor reaction counted as a response to light.

**The influence of the intensity of light on the time of reaction.**— Several animals were tried in each of three intensities of light. For the lowest intensity weak daylight, obtained by drawing the shade of the window in front of the dish, was used, for the medium intensity ordinary daylight, and for the strong intensity direct sunlight. Tables III, IV, and V contain the results of the experiments made. In each table the figures indicate the average for ten reactions, and their average variability.

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In Table III, animal No. 1 shows a regular decrease in the time of reaction and likewise of variability with the increase in light intensity. For it the statement, the stronger the stimulus the shorter and less variable the reaction time, is true. Animal No. 2, however, reacted to the medium light little quicker than to weak light. The variability, moreover, is extremely great, 7.76 seconds. The meaning of these facts is easily discoverable, for when we refer to the record of individual reaction times, we find that three very slow reactions are included in the series; hence, the unexpectedly long average reaction time and the high variability. Clearly this result, because of the great difference in variability, is not directly comparable with that obtained with animal No. 1. Careful examination of thousands of reaction times, and of the conditions determining them, in so far

TABLE IV.

REACTION TIMES TO THREE INTENSITIES OF LIGHT.

| Weak daylight. | | Daylight. | | Sunlight. | |
|---|---|---|---|---|---|
| Mean. | Mean variation. | Mean. | Mean variation. | Mean. | Mean variation. |
| 8.8 | 1.62 | 8.3 | 3.35 | 7.7 | 2.52 |
| 7.2 | 3.21 | 5.2 | 1.63 | 3.9 | 0.55 |
| 11.2 | 3.67 | 6.8 | 1.89 | 4.8 | 1.92 |
| 8.8 | 2.63 | 6.0 | 1.58 | 4.3 | 1.09 |
| 11.0 | 4.67 | 8.7 | 3.49 | 6.5 | 1.93 |
| Gen. av. 9.4 | 3.16 | 7.0 | 2.39 | 5.5 | 1.60 |

as observable, shows that approximate equality in variability is the only safe basis for direct comparison of reaction times. By equality is meant proportionate, not absolute equality. If for one animal the variability of an average reaction time of ten seconds is four seconds, for another individual, the conditions remaining constant, the variability of a reaction time of five seconds should be about two seconds in order to make direct comparison valuable. The general averages of Table III are curiously misleading. They indicate a decrease in the time of reaction to medium light as compared with weak light, and also as compared with strong light. This fact

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in itself is not surprising in view of what we have already learned concerning the inhibitory effects of sunlight. But all the results which show anything approaching proportionate equality in variability[1] indicate a shorter time of reaction for sunlight than for either of the other intensities. In Table IV, for instance, the results of the reactions of five animals is given for the three intensities of light. The results are not for the same individuals for each intensity, as in Table III, but for animals taken at random.

Comparison of the averages of this table show: (1) decrease in reaction time with increase in intensity of illumination, and (2) approximate proportionate equality in variability. In no case is the

TABLE V.

REACTION TIME TO DAYLIGHT AND TO SUNLIGHT.

| Animal. | Daylight. | | Sunlight. | |
|---|---|---|---|---|
| | Mean. | Mean variation. | Mean. | Mean variation. |
| 1 | 8.3 | 3.35 | 7.7 | 2 52 |
| 2 | 5.2 | 1.63 | 3.9 | 0.55 |
| 3 | 7.0 | 3.44 | 4.8 | 1.92 |
| General av. | 6.8 | 2.81 | 5.5 | 1.70 |

latter much more than one-third of the reaction time. A variability in excess of one-third of the reaction time is indicative of irregularities in the series of reactions due to lack of uniformity of conditions.

The averages of Table III are misleading because of the exceptional reactions of some of the animals. That weak light usually causes a slower reaction than ordinary daylight is proved by the results of Tables III and IV. That sunlight does not at first cause a slower reaction than daylight is proved by the results of Tables IV and V. In the latter table the times stand 5.5 to 6.8. The variability of the sunlight reaction time is 1.70, that is, thirty-one per cent of the reaction time. The variability of the daylight reaction time is 2.81, that is, forty-one per cent of the reaction time. This increase in

[1] The ratio of variability to reaction time being about the same for different individuals.

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variability with decrease in strength of the stimulus is precisely what all previous reaction time work leads us to anticipate.

The conclusions to be drawn from the results of Tables II to V are: (1) Gonionema's reaction time to light becomes shorter as the stimulus increases in strength; (2) absolute variability of reaction time also decreases as the stimulus increases; (3) sunlight usually causes quicker reaction than daylight, and (4) a variability of more than thirty to forty per cent of the reaction time is indicative of inconstancy of conditions.

**Relation of reaction time to light to size.** — Small Gonionemata are more active than large ones. Counting the number of contractions of the bell per minute for a period of five minutes in case of animals of different sizes yielded the following results:

Animals of 20 mm. in diameter gave an average of about 15 beats per minute.
" " 15 " " " " " " " " 22 " " "
" " 10 " " " " " " " " 30 " " "
" " 5 " " " " " " " " 51 " " "

There are of course noteworthy exceptions to the rule, but undoubtedly activeness varies inversely as the size.

Further evidence of the relation of size to activeness and to the time of reaction was furnished by observations made on Gonionemata which were left in covered dishes over night, and whose reaction time to light was noted when they were uncovered in the morning. The average time of nine small animals under such conditions was thirteen seconds, whereas for eight large animals it was 21.8 seconds. A large number of observations gave averages of about fifteen seconds for the small individuals (5–12 mm.), and about twenty seconds for the large ones (12–20 mm.).

We have now to ask whether this relation of size to time of reaction to light appears in the results of the reaction time experiments already described. Ten reactions for each of twelve Gonionemata, ranging in size from 5 to 10 mm. in diameter, gave an average reaction time of 7.3 seconds. And a like number of reactions for each of eighteen individuals, which ranged from 11 to 16 mm., gave an average reaction time of eleven seconds. The smaller individuals are much more active and usually respond to stimulation by light more quickly.

**Relation of reaction time to light to pigmentation.** — Gonionemata usually have granular pigment in the gonads, tentacles, margin and

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radial canals, but the quantity of this pigment varies greatly among individuals; some are so light as to be almost transparent when in water, others are yellowish, greenish brown, or even dark brown. The question may therefore be asked, are heavily pigmented individuals detectably different from those which lack pigment in their reactions to light?

The average reaction time for thirteen very lightly pigmented individuals (ten reactions for each) was 7.9 seconds, and for the same number of heavily pigmented animals 7.2 seconds. This difference although not great is doubtless indicative of the physiological importance of pigmentation. Moreover, comparison of all the results shows that the dark individuals are quicker, size, sex, temperature, etc., being considered, than are light ones. This fact came out most strikingly in the general tests of light sensitiveness made by placing ten Gonionemata in a large dish, leaving them covered over night, and then noting their positions and time of reaction when they were exposed to light in the morning. By this method a group of ten light animals was found to react in 22.5 seconds under the conditions which gave for a similar group of dark individuals a reaction time of fifteen seconds. As in case of large and small animals, an individual is often found which gives contradictory results, so in the case of light and dark individuals one occasionally meets with marked exceptions to the rule that dark animals react more quickly than light ones.

**Relation of reaction time to light to sexual condition.** — There can be no doubt that the reaction of Gonionema to light is largely dependent upon its sexual condition. Individuals with ripe gonads if brought into the laboratory can be made to discharge their sexual products in from thirty minutes to two hours by putting them in the dark.[1] In earlier experiments attention was called to the fact that animals with heavy gonads when exposed to direct sunlight become negative to it, and swim toward the bottom of the dish sooner than do immature individuals. The greater sensitiveness of sexually ripe individuals is thereby indicated.

Reaction-time results of this paper show no pronounced differences between males and females.

**The influence of changes in temperature on the time of reaction.** — A sudden increase in temperature shortens the reaction time, a sudden

[1] See PERKINS on the Development of Gonionema murbachii, Proceedings of the Academy of Natural Science, Philadelphia, 1902, liv, pp. 755-756.

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decrease lengthens it, or causes complete inhibition of reaction. The purpose of the following experiments was to ascertain the effects of a gradual change in temperature upon the photic reactions of Gonionema.

A single animal, for the experiments, was placed in a small earthenware dish containing water, and this dish was placed in the water of a larger vessel. Then, by the application of heat to the outer vessel, the temperature of the water in which the medusa swam was raised regularly at the rate of one degree in ten minutes. By placing ice in the water of the outer vessel, the temperature was lowered at the same rate.

For each animal used the reaction time to daylight at the temperature of the sea-water was first determined. Then as the temperature was gradually changed one reaction per minute was taken, thus, allowing for irregularities of reaction and time to read and record

TABLE VI.

REACTION TIME TO DAYLIGHT AT DIFFERENT TEMPERATURES.

| At 17° C. | | At 19° C. | |
|---|---|---|---|
| Mean. | Mean variation. | Mean. | Mean variation. |
| 8.7 | 3.22 | 8.5 | 1.60 |
| 14.0 | 3.74 | 8.1 | 0.89 |
| 10.0 | 2.04 | 6.3 | 0.64 |
| 12.2 | 3.30 | 5.9 | 1.59 |
| Gen. av. 11.2 | 3.00 | 7.2 | 1.18 |

results and take the temperature readings, it was possible to get at least five reaction time readings while the temperature changed one degree. As in the previous experiments, the reactions were given to increase in light intensity caused by uncovering the dish at regular intervals. These experiments were performed during August and September, and as the temperature of the water from which the Gonionemata used were gotten fell four or five degrees during that period, an opportunity was given for noting whether the reaction time of the animals changes with this environmental change. In Table VI,

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the reaction time to daylight is given for four animals taken from water about 17° C., and for the same number of animals taken from water of 19° C. temperature. At the higher temperature the

TABLE VII.

THE EFFECT OF GRADUAL INCREASE IN TEMPERATURE ON REACTION TIME TO LIGHT.

| Temperature. | Reaction time. | Mean variation. |
|---|---|---|
| | secs. | |
| 19° (normal) | 9.1 | 1.40 |
| 20° | 7.8 | |
| 21° | 6.5 | |
| 22° | 6.2 | |
| 23° | 6.3 | |
| 24° | 5.9 | 0.94 |
| 25° | 5.7 | |
| 26° | 5.2 | |
| 27° | 4.6 | |
| 28° | 3.8 | |
| 29° | 3.7 | |
| 30° | 3.5 | 0.48 |
| 31° | 2.7 | |
| 32° | 2.6 | 0.32 |
| 33° | 4.8 | |
| 33° | 6.9 | 1.51 |
| 33.5° | 25.6 | failure to respond. |

reaction time is 7.2 seconds, the variability only 1.18; at the lower temperature the time is 11.2 seconds, the variability 3. It is significant that such a great increase in variability accompanies the lower temperature. This variability, it may be remarked, is greater in proportion to the time of reaction as well as absolutely. For the 19° reactions, the ratio of variability to reaction time is 1 : 6.1 (variability of 16+ per cent), for the 17° reactions the ratio is 1 : 3.7 (variability of 27− per cent). This shows that temperature is an

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important factor in the determination of the variability of reaction time. That strength of stimulus is one of the most important determinants is already well known,[1] but for the interpretation of reaction time results it is of importance to discover precisely what other conditions need to be considered. By such studies as this I hope to be able to discover the relations of the chiefly significant environmental conditions to the time and variability of an animal's reactions.

Table VII presents the results of series of experiments with three Gonionemata. Each animal was placed in a dish of water of normal (sea-water) temperature, the reaction time to daylight determined, and the temperature then raised at the rate of one degree in ten

TABLE VIII.

THE EFFECT OF GRADUAL DECREASE IN TEMPERATURE ON REACTION TIME TO LIGHT.

| Temperature. | Reaction time. | Mean variation. |
|---|---|---|
| | secs. | |
| 19° (normal) | 8.2 | 1.82 |
| 18° | 12.2 | |
| 17° | 15.9 | 4.50 |
| 16° | 17.3 | |
| 15° | 14.6 | 3.10 |
| 14° | 23.0 | |
| 13° | 32.3 | 15.80 |
| 12° | most individuals irresponsive. | |

minutes until reactions to light ceased. Each result (*e.g.* 20°, 7.8 seconds) in the table is the average time for fifteen reactions, five for each of three animals, which were given while the temperature was increased one degree. This table indicates a gradual shortening of the time of reaction until 32° is reached. Between 32° and 33° the reactions are quickest; but if an animal be exposed to this temperature for a few minutes, the reactions rapidly become slower and soon cease. At 33° an increase in reaction time begins, and before 34° is reached reactions usually fail entirely, and the animals soon

[1] YERKES: Harvard psychological studies, 1903, i, pp. 579–638.

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die unless removed to a lower temperature. In a few cases the variability has been given in Table VII in order to show the decrease in variability which is correlated with the quickening of reaction time due to increase in temperature. The shortest reaction time, that at 32°–33°, is also the least variable. Gradual increase in temperature up to a certain point shortens the reaction time of Gonionema to light; what is true of decrease in temperature?

The apparatus employed in the study of the effects of increase in temperature was here used again, with ice in the outer vessel. The averages of Table VIII are based on the reactions of four animals. The reaction time usually lengthens as the temperature decreases, but in certain cases a shortening of the time at first is followed by a rapid lengthening. In these exceptional cases cold seems to act as a stimulus, at first, in much the same way as does heat.

Decrease in temperature, as is shown by the results of Table VIII, gradually increases the time of reaction until reactions finally cease entirely at a temperature of from 12° to 14°. An animal which has been exposed to low temperature for hours often reacts to daylight when at a temperature of 9°–10°.

Increase of temperature is accompanied by greater activeness of Gonionema until 33° is reached. At that point the animals begin to weaken, contraction becomes irregular and partial, and the margin takes the form of a square instead of preserving its usual circular form. Apparently heat causes the contraction of the portions between the radial canals. The manubrium usually expands fully at from 30° to 33°, and becomes irresponsive to mechanical or photic stimuli.

The effects of decrease of temperature on Gonionema in contrast with those of increase are a general lessening of activeness and a tendency toward quiescence in a state of expansion. At from 8° to 12° most individuals become entirely irresponsive to ordinary stimuli. They do not appear to be weakened or in any way injured by the low temperature, as in case of high temperatures, for after removal from ice water to water of ordinary temperature they soon recover their usual activeness and respond normally. Heat causes contraction; cold causes expansion.

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## Localization of Sensitiveness of Gonionema to Photic Stimuli.

Early in the course of this experimental study it became evident that Gonionema's reaction to light partly depends upon its position with reference to the source of light. Usually the animals after a period of activity come to rest with the apex of the bell on the bottom of the experiment dish; this position we may know as the "bell-down" position. In this position the under surface of the margin,

TABLE IX.

Series of Reaction Times Showing the Influence of Position.

\+ indicates reactions when the bell was up. – indicates reaction when the bell was down. ⊥ indicates reaction when the bell was on edge.

| | No. 1, 14 mm. Dark color. Temp. 17.4°. | No. 2, 11 mm. Light color. Temp. 17.4°. | No. 3, 18 mm. Light color. Temp. 21°. |
|---|---|---|---|
| 1 | 8.4– | 5.8– | 7.3– |
| 2 | 10.4– | 6.3– | 10.8– |
| 3 | 13.0– | 17.0+ | 7.4+ |
| 4 | 13.2– | 6.9– | 6.8– |
| 5 | 15.3– | 10.8+ | 5.8– |
| 6 | 14.6– | 9.7– | 6.9– |
| 7 | 35.8+ | 7.3– | 29.5+ |
| 8 | 65.0+ | 7.2– | 8.0– |
| 9 | 9.6– | 8.3– | 5.9– |
| 10 | 22.2⊥ | 8.0– | 25.2+ |
| 11 | 8.8– | 7.1– | 29.7+ |
| 12 | 13.5– | 8.7– | 8.3– |
| 13 | 10.9– | 6.2– | 13.2– |
| 14 | 11.7– | 7.2– | 34.7+ |

the inner surface of the bell, and the manubrium are exposed to the light. Sometimes, however, an animal settles down with the apex of the bell uppermost, this, the "bell-up" position, does not expose so much of the margin to the action of light as does the "bell-down"

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position. Gonionemata which have been left undisturbed in weak light for a few hours are usually found resting "bell-up."

Three series of reaction times, given by individuals differing in size and color, are presented as evidence of the relation of reaction time to the animal's position with reference to the light. Reactions when the animal was resting "bell-up" are followed by a + sign; reactions when the animal was resting "bell-down," by a – sign. The results of Table IX indicate that the "bell-up" position is seldom assumed, and that when in this position the medusæ react much more slowly to light than when resting "bell-down." An examination of all records obtained shows that Gonionema is much more likely to come to rest "bell-up," in the presence of some disturbing condition, such as high or low temperature, than under usual conditions, while in almost all cases the reaction time is much shorter for the "bell-down" position.

This evident dependence of reaction time to light on the position of the animal necessitated the rejection of all "bell-up" reactions from averages, except where the results were used for the special purpose of showing the influence of position. The necessity is evident when one examines such series as those of Table IX. There, in case of No. 1, three exceptional ("bell-up" +) reaction times, if included in the series, increase the average greatly, and cause the result to be quite misleading.

Having noted the importance of position, we have now to ask why the reaction time for the "bell-down" position is so much the shorter? That the difference is caused by greater ease in movement is unlikely; that the "bell-down" position is that in which the animals most frequently react to stimulation by light, and, therefore, the more favorable is possible; yet, one first of all is inclined to believe that a difference of sensitiveness is at the bottom of the matter. If the "bell-up" position causes certain organs or parts of Gonionema which are especially sensitive to light to be covered, whereas the opposite position leaves them freely exposed to the stimulus, a sufficient cause for the difference in reaction time appears.

With a view to determining what portions of the medusa are sensitive to light, and at the same time solving the problem of the difference in reaction time above noted, certain experiments now to be described were tried.

First, the effect upon photic reaction time of excising all the

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tentacles of an individual about 2 mm. from the margin was noted. This experiment seemed worth while because of the evident importance of the tentacles as sensory, and as orientation organs. The question is, are they sensitive to light in any marked degree, and does the medusa when deprived of them react otherwise to light than the normal individual? In many cases the operation of tentacle excision causes a shock from which the animal never fully recovers, but in others the reactions to light continue uninterruptedly.

The reactions of a 16 mm. individual are presented in Table X. For this individual the normal reaction time before the operation was

TABLE X.

REACTION TO LIGHT AFTER REMOVAL OF TENTACLES.

Reaction time of normal animal, 4.7 seconds. Reactions taken ten minutes after removal of tentacles. Missed indicates failure to react within one minute.

| | | | | | |
|---|---|---|---|---|---|
| 1 | Missed + | 11 | 2.2– | 21 | 20.8+ |
| 2 | 6.2– | 12 | 6.2– | 22 | 5.4– |
| 3 | 15.6– | 13 | 3.2– | 23 | 1.4– |
| 4 | 9.8– | 14 | 1.8– | 24 | Missed + |
| 5 | 8.7– | 15 | 3.8+ | 25 | 4.8+ |
| 6 | 2.0– | 16 | 1.0– | 26 | 2.2– |
| 7 | 1.2– | 17 | 13.4+ | 27 | Missed + |
| 8 | 1.6– | 18 | 1.1– | 28 | 1.1– |
| 9 | 1.8– | 19 | 1.0– | 29 | 2.1– |
| 10 | Missed + | 20 | 1.2– | 30 | 44.1+ |

| | | Variability. |
|---|---|---|
| General average of all reactions (26) . . . . . . . . | 6.3 | 5.74 |
| Average of all "bell-up" reactions (+) (5) . . . . | 17.4 | 12.06 |
| Average of all "bell-down" reactions (–) (21) . . . | 3.4 | 2.77 |

4.7 seconds. After the operation the "bell-up" reactions were exceedingly long, the "bell-down" reactions unusually short. This result, which was gotten also with other individuals, seems to indicate that when a normal animal is resting "bell-up," the tentacles are stimulated by light sooner than are the organs of the under side of

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the margin; hence, the animal reacts more quickly with the tentacles than without them. When the tentacles are removed, the "bell-down" reaction time is slightly quicker, because of the increased irritability of the margin. The results show: (1) that the tentacles are sensitive to light, and (2) that they are not responsible for the difference in the reaction times to light for "bell-up" and "bell-down" positions.

Experiments with the marginless bell prove conclusively that the bell itself is irresponsive to light. In these tests, light stronger than direct sunlight and lamplight was not used. The marginless bell reacts to *strong* mechanical, chemical, and thermal stimuli, but never, so far as my observation goes, to photic stimuli. It thus appears that the general surface of the bell is insensitive to light.

It may here be mentioned that animals whose tentacles were removed by pulling them off in such a way as to destroy the marginal bodies never reacted to light. The manubrium of such individuals seems to be sensitive, but it does not initiate bell contractions.

We are now confined in our search for the special sense organs for photic stimuli to the margin of the bell. Margins when excised at a distance of four or five millimetres from the edge in some cases contract closely and fail to respond with any regularity to daylight; others, however, remain expanded, except when stimulated, and react uniformly. The facts brought out by experiments on excised margins are: (1) that the margin, containing the marginal bodies, is exceedingly sensitive to light, if quickness of reaction is to be taken as evidence of sensitiveness, (2) that the margin, unlike the normal animal, responds regularly to decrease in light intensity as well as to increase, (3) that the upper surface of the margin is less sensitive than the lower surface, (4) there is some evidence that the "bell-up" margin reacts more quickly to decrease in light intensity than to increase, whereas the reverse is sometimes true for the "bell-down" margin.

The "bell-up" margin reacts to increase in light intensity very seldom, but to decrease almost uniformly. When the time of reaction (13.6 seconds, Table XI) for increase in intensity when the margin is in the "bell-up" (+) position, is compared with the reaction to increased intensity when the margin is in the "bell-down" (–) position, account should be taken of the frequency of reaction as indicated at the bottom of Table XI, as well as of the time. In these experiments, increase of intensity was obtained by uncovering the

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experiment dish and exposing the animal to daylight, decrease by covering the dish and thus cutting off a part of the light.

The excised margins always tend to maintain the "bell-up" position, and have to be turned over repeatedly during experiments which require the "bell-down" position. In this we have additional evidence of the greater sensitiveness of the under surface of the margin.

TABLE XI.

REACTION TIME OF EXCISED MARGINS TO INCREASE AND DECREASE OF LIGHT INTENSITY WHEN IN "BELL-UP" AND "BELL-DOWN" POSITIONS.

| Animal. | When in "bell-up" (+) position. | | | | When in "bell-down" (—) position. | | | |
|---|---|---|---|---|---|---|---|---|
| | Increase in intensity of light. | | Decrease in intensity of light. | | Increase in intensity of light. | | Decrease in intensity of light. | |
| | Reaction time. | Variability. | Reaction time. | Variability. | Reaction time. | Variability. | Reaction time. | Variability. |
| 1 | 15.2 | 20.07 | 1.1 | 0.29 | 14.2 | 16.67 | 1.3 | 0.44 |
| 2 | 12.1 | 6.50 | 2.8 | 1.28 | 16.3 | 13.76 | 9.0 | 3.10 |
| 3 | .. | .. | .. | .. | 3.3 | 2.50 | 9.7 | 4.90 |
| Gen. av. | 13.6 | 13.28 | 1.9 | 0.78 | 11.3 | 10.98 | 6.7 | 2.85 |
| | Reaction to about 30% of stimuli. | | Reaction uniformly. | | Reaction to about 90% of stimuli. | | Reaction almost uniformly. | |

Certain observations concerning the reactions of normal Gonionemata to light bear on the same point. One animal, a 26 mm. female with ripe gonads, reacted to increase in light intensity uniformly when "bell-up" (+) in from eight to fifty seconds, and when "bell-down" (—) in from five to ten seconds. To decrease in intensity this individual reacted in about 25 per cent of the experiments, and of this 25 per cent almost all were "bell-up" reactions. These results are entirely in harmony with those obtained with excised margins. The above individual when subjected to strong lamp-light reacted uniformly to increase in intensity whether "bell-up" or "bell-down." It never came to rest in the light otherwise than "bell-up," and as long as it was turned over by the experimenter in

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such fashion as to prevent it from taking that position, it continued swimming. Furthermore, the reactions in the "bell-up" position were very much slower than those in the "bell-down" position. The latter could be obtained only by turning the animal over, and then cutting off the light immediately.

As evidence of the greater sensitiveness of the lower as compared with the upper surface of the margin, as well as of the commonness of reaction to increase but not to decrease of light intensity, the following results obtained with an exceedingly dark individual 17 mm. in diameter are also presented. This animal always came to rest "bell-up" when in daylight, and just as regularly changed to the "bell-down" position during the time that the dish was darkened. When in a shaded portion of the dish it usually assumed the "bell-down" position. To increase in light it often reacted so quickly that it was impossible satisfactorily to measure the time with a stop watch. In many cases the time was evidently less than one second. To decrease in light intensity, it reacted once in 4.3 seconds when "bell-up," but thereafter twenty repetitions of the stimulus at regular intervals of a minute when the animal was sometimes "bell-up," sometimes "bell-down," failed to call forth a reaction. These individual reactions bring out with unusual clearness what has been found to be true in general for Gonionema.

In view of the above results we may conclude that the margin of the bell contains organs which are especially sensitive to photic stimuli; and since destruction of the marginal bodies destroys responses to light the probability is strong that they are the special organs of photic stimulation.[1] They are heavily pigmented organs so placed that light can affect them directly only when the animal is "bell-down," hence the slowness of reaction when an animal is in the "bell-up position.

## Summary and Conclusions.

1. In its natural habitat Gonionema is negative to intensities of light greater than ordinary daylight. When disturbed, it leaves its attachment to submerged sea-weeds and swims surfaceward. If it reaches the surface while swimming, it turns over as soon as the apex of the bell emerges from the water, ceases contracting the bell,

[1] For a description of the Nervous System of Gonionema, see the preliminary paper of Miss I. H. Hyde: Biological bulletin, 1902, iv, pp. 40–45.

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and sinks passively with fully expanded bell and tentacles. Although in moving surfaceward the medusa swims toward the source of light, the direction of movement is not determined by light. The turning reaction, inhibition of activity, and expansion which mark the appearance of an animal at the surface of the water although not entirely due to light are, at least in nature, uniformly associated with an increase in the intensity of illumination.

2. Under experimental conditions, Gonionema moves toward the source of light, *i.e.*, is positively phototactic. It comes to rest in the darkest portion of the vessel, and is therefore negatively photopathic to ordinary intensities of light.

3. Direct sunlight strongly stimulates the animals. When first exposed, they swim surfaceward toward the light, but after a period of exposure, dependent upon the intensity of the light and the size, pigmentation, and sexual condition, they become negative to the light and swim downward, and into the shaded regions of the vessel.

4. When exposed to sunlight in a dish one end of which is shaded, they soon gather in the shaded portion, and at least eighty per cent of them are always found there. Although when an animal enters the shaded part of the vessel its activeness decreases, and it frequently comes to rest, this does not suffice to explain the gathering of the animals in that region. Observation shows that in most cases as soon as an animal passes from the shade into the sunlight it ceases swimming, turns over, and sinks with expanded organs, just as it does in its natural environment on reaching the surface of the water. Apparently increase of light in this case has an inhibitory influence. As soon as the medusa reaches the bottom of the vessel, it again contracts, but this contraction is of such a nature as to turn the animal toward the shaded region, so that it again swims back into that portion of the vessel.

5. Light, like other stimuli, determines the direction of movement of Gonionema through the unequal contraction of the bell due either to localized or unequal stimulation. That portion of the bell most strongly stimulated reacts by a more forceful contraction than the remainder of the bell, and thus tends to turn the animal away from the region of stimulation.

6. Electric stimulation most clearly demonstrates the "directive influence" of stimuli because it can be applied without interfering with the animal's movements by direct contact. A Gonionema rest-

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ing with the apex of the bell uppermost ("bell-up" position), when stimulated at a certain point on the margin by the approach of electrodes, reacts by a contraction of the bell, which, because it is more forceful at the point of stimulation, forces the side of the bell stimulated upward most rapidly, thus tending to remove the animal from the region of the stimulus, instead of taking it toward it. A "bell-down" individual reacts to the same kind of stimulus by a contraction which, because of its inequality, forces the side stimulated downward most rapidly, thus causing the bell to turn away from the stimulus.

7. That Gonionema is able to direct its movements by means of such unequal contractions as are here mentioned, is proved by the fact that animals which at first swim to the surface in direct sunlight later turn over before reaching the surface.

8. Increase in light intensity uniformly causes a motor reaction in quiescent individuals, and the inhibition of movement in active individuals.

9. Decrease in light intensity usually causes the inhibition of movement in active animals, but rarely does it act as a stimulus to activity in case of resting animals.

10. Strong light is injurious to Gonionema, and a few hours' exposure is commonly fatal.

11. To the increase in light intensity resulting from the uncovering of the vessel containing it, Gonionema uniformly gives a locomotor contraction of the bell. The time of this reaction in daylight is from five to ten seconds.

12. The reaction time to light decreases with increase in the intensity of the light. For weak daylight it is 9.4 seconds, and for ordinary daylight 7.0 seconds.

13. The time of reaction to sunlight is 5.5 seconds as compared with 7.0 seconds for daylight.

14. The variability of the reactions to light is usually about one-third of the reaction time. A greater variability than this in ordinary reactions is indicative of some exceptional condition which should be taken into account.

15. The activeness of Gonionema varies inversely as the size of the animal.

16. Small animals, other things being equal, react more quickly to light than do large ones.

17. Heavily pigmented individuals react more quickly than do those which are lightly pigmented.

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18. Sexually mature individuals appear to be more sensitive to light than immature or senile animals.

19. Increase in temperature gradually shortens the reaction time to light. At 33° the reaction time is about 2.5 seconds, with a variability of only 0.4 of a second. Beyond this temperature the reaction time rapidly lengthens, and at 34° the animal soon perishes.

20. Decrease in temperature gradually lengthens the reaction time to light, until reaction fails entirely at 10°–12°.

21. Gonionemata, when "bell-up" in the water, react to light falling upon them from above much less quickly than they do when "bell-down" in position. This, together with certain results obtained with excised margins, proves that the under surface of the margin is the portion of the medusa especially sensitive to light.

22. Since destruction of the marginal bodies renders the margin irresponsive to light, we conclude that these bodies are the sense organs for photic stimuli.

23. The marginless bell is entirely irresponsive to light intensities not greater than that of direct sunlight.

24. Excised margins in many cases react to either increase or decrease of light intensity in much less time than do normal animals. They differ markedly from the latter in the uniformity of their reactions to decrease in light.

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# EXPERIMENTS IN ARTIFICIAL PARTHENOGENESIS.

By E. P. LYON.

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A NUMBER of American investigators have successfully repeated Loeb's experiments on artificial parthenogenesis. In Europe, Delage has taken up the work with marked success. Most of those who have worked on the problem at Naples have, on the contrary, reported negative or indifferent results. During the past autumn I spent some weeks at the Naples Zoölogical Station; and at the suggestion of Professor Loeb, who thought that one familiar with the material and conditions at Woods Hole might be better able to cope with the difficulties at Naples, I made experiments in parthenogenesis with a variety of forms and methods.

## Precautions.

In all my experiments the dishes and instruments were sterilized by boiling. All sea-water used was heated in glass to 60°–70° C., and filtered. After cooling, enough distilled water was added to make up the loss due to evaporation. The practically sterile sea-water thus prepared was thoroughly shaken with air. The animals used were thoroughly washed under a violent stream of tap-water. The hands of the operator were washed in very hot fresh water.

## Experiments on Echinoderms.

1. **Arbacia.**— Arbacia pustulata found at Naples resembles, at least superficially, Arbacia punctulata of the American coast. Physiologically it differs from our species in a greater natural tendency of the eggs to divide parthenogenetically without any treatment whatsoever. As a rule, the eggs did not begin to change for from twenty to twenty-four hours. Then they began to segment rapidly, and it was not unusual to find, a few hours later, that 80 per cent or 90 per cent of the eggs were in various conditions, from two cells to forms that appeared to be irregular and pathological morulæ. I say "appeared to be" because I made no tests to show that the loose masses were



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really composed of cells, nor did I watch their production from the start. These forms were very numerous, and were similar to those pictured by Ariola.[1] It will be remembered that he inadvertently used solutions which Loeb had employed for another purpose, but not for artificial parthenogenesis. Ariola's failure was probably due to this error. And the forms he figures are apparently not different from those found in my experiments among untreated eggs, as well as among those which had been subjected to treatment, but not to the exact conditions for successful parthenogenetic development. These forms easily fell apart, so that often little was left of a culture of eggs but a mass of debris.

In some experiments in the untreated controls, a few cleavage forms were found which appeared more nearly normal; and I often examined my controls with extreme care to see whether swimming larvæ were produced. On two occasions two or three weak ciliated fragments were found; never any normal larvæ. That normal parthenogenesis may sometimes occur in Arbacia pustulata seems to me only remotely possible. Indeed, the possibility that the ciliated fragments were not derived from the eggs at all should not be lost sight of, although it seems to me a remote conjecture, considering the manner of preparation of the water and eggs used. At any rate, I am sure that accidental impregnation with sperm is not to be considered in these cases. The controls were most carefully examined at times when fertilized eggs would be in early cleavage stages and easily detected, and not one was found.

In spite of the above-mentioned natural tendency toward development, artificial parthenogenesis was more difficult to produce in Arbacia pustulata than in the Woods Hole species. There was greater variability in the material furnished from day to day. This may be due to the perennial breeding habit of Arbacia pustulata as contrasted with the limited period of Arbacia punctulata. It is harder to find females with eggs in exactly the right stage. Under the proper conditions, however, unequivocal results were obtained.

An easy method of getting eggs unmixed with body liquids or intestinal contents was found in Arbacia pustulata. If the animals were held under the fresh-water tap for a minute or two (as was done in washing them) and then left in the air for about a minute, they shed their eggs in great quantities. With a pipette the eggs were washed into sterile sea-water. The males, also, when similarly treated,

[1] Ariola: Atti Società ligure, Genoa, 1901, xii, p. 12.

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shed their sperm, but a somewhat longer time of exposure to the water and air was required. The eggs thus obtained fertilized normally with the sperm, and excellent cultures of normal plutei were obtained. This method of obtaining the eggs was used in almost all the experiments on Arbacia. No experiments were made to ascertain the nature of the stimulus which caused the eggs to be shed. The temperature of the Naples hydrant water was noticeably low. Cold, mechanical stimulation and the action of fresh water are possible explanations.

Hydrochloric acid has been found by Loeb to be an efficient reagent for causing artificial parthenogenesis in star-fish. He found it did not succeed in Arbacia punctulata. But, strangely enough, it is one of the best reagents I found for Arbacia pustulata. Usually 2, 3, 4, 5, 6, and 7 c.c. of a solution of $\frac{m}{10}$ hydrochloric acid in sea-water were added respectively to dishes containing 100 c.c. of sea-water. Eggs immersed in these solutions were taken out at intervals of from two to fifteen minutes. It was found advantageous to wash away the excess of acid by dropping the eggs from a pipette into test-tubes filled with sea-water. The eggs sank slowly through the water, which was then drawn off. More sea-water was added, and the eggs poured into dishes containing about 100 c.c. Some of the best results were obtained from 2 c.c. acid in 100 c.c. of sea-water, ten to fifteen minutes' exposure; 3 c.c. acid, seven to twelve minutes' exposure; 4 c.c. acid, nine minutes' exposure; 7 c.c. acid, five minutes' exposure. In general, a short exposure to stronger acid might be said to give equal results with longer exposure to weaker solutions; but the exact duration for strong solutions was harder to ascertain. In the best experiments perhaps 10 per cent of the eggs developed to swimming larvæ. Many of these swam up to the top of the liquid, just like larvæ from fertilized eggs. They formed fully developed plutei, which lived as long as individuals produced from fertilized eggs and kept under the same conditions.

For extracting water from eggs according to Loeb's first successful method for producing artificial parthenogenesis, I used potassium chloride and sodium chloride. These two salts have been found by Loeb[1] to be best adapted to this purpose, although, of course, sugar and other non-electrolytes may be used. Potassium chloride, in my hands, gave the best results. Solutions containing 10 to 16 c.c. of $2\frac{1}{2}\,m$ potassium chloride in 100 c.c. of sea-water and exposures of

[1] J. Loeb: Archiv für Entwickelungsmechanik der Organismen, 1902, xiii, p. 482.

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one to two hours were used. These are the strengths of solutions and approximate times used by Loeb. The Naples material is not different markedly from that at Woods Hole, so far as parthenogenesis by this method is concerned. Normal plutei were produced.

While I was working at Naples, Delage[1] published his first paper on parthenogenesis produced by carbon dioxide. He succeeded with star-fish, and calls carbon dioxide the ideal reagent, but failed with sea-urchins. I tried his method on the Naples forms. Siphons of sterile sea-water were charged with the gas by the local mineral water company. This water was drawn off, and after the active effervescence ceased, solutions of one half sea-water and one half carbon dioxide water, one third sea-water, and two thirds carbon dioxide water, etc., were prepared. The eggs were exposed to these solutions for various times from three minutes to several hours. Six to twenty minutes seemed best.

The undiluted carbon dioxide solution acted like too strong acid. The pigment of the eggs was dissolved out, and the eggs became sticky and agglutinated. Weaker solutions caused very marked cell division. Within three or four hours as many as 90 per cent would sometimes begin development. But no active larvæ were produced from Arbacia eggs.

Shaking failed to produce larvæ from Arbacia eggs, though eggs that had been mechanically agitated showed cell division earlier and in larger amount than the unshaken control.

During the last weeks of my work (November) it became more difficult to obtain artificial parthenogenesis in Arbacia; and perhaps the material is still more difficult to work with in winter, which is the season most workers have used it. At the same time difficulty in obtaining natural fertilization was experienced. In some cases I was able to cause artificial parthenogenesis in eggs which would not fertilize with sperm. Usually, however, eggs that failed to develop by one method also failed by the other.

2. **Strongylocentrotus.** — The species used at Naples was Strongylocentrotus lividus. It did not give good results with hydrochloric acid, though cell division was accelerated. On the other hand, potassium chloride gave better results than with Arbacia. Thousands of fine plutei were produced in a dozen or more experiments, and some

[1] Delage: Comptes rendus de l'académie des sciences, 1902, cxxxv, p. 570 and p. 605. See also, Archives de zoologie expérimentale et générale, 1902, 3me serie, x, p. 213.

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were alive when I left Naples twenty-two days after the experiment began. The best results were obtained by adding from 10 to 14 c.c. of $2\frac{1}{2}$ *m* potassium chloride to 100 c.c. of sea-water. The eggs were left in these solutions from one to two hours, one and one half hours being, perhaps, more usually satisfactory than any other time.

Sodium chloride did not give so good results as potassium chloride, although plutei were obtained by its use.

In order to keep these or any other parthenogenetic larvæ alive for any length of time, it was necessary to pick them out from the dishes containing disintegrative fragments of eggs which failed to develop, and place them in clean sea-water. But larvæ produced by natural fertilization require the same care if undeveloped eggs or dead larvæ are present in any quantity.

Carbon dioxide gave better results with Strongylocentrotus than with Arbacia, but it is far from the ideal reagent for either species. It stimulates cell division very markedly. A few larvæ were produced from eggs of Strongylocentrotus exposed from two to eight minutes to a solution consisting of one half to five sixths carbon dioxide water and one half to one sixth sea-water. Some of these became plutei.

Delage believes that carbon dioxide is a specific reagent for star-fish eggs, that it does not act by its acid property, and fails altogether with sea-urchins, including Strongylocentrotus. It is worthy of notice, perhaps, that in my experiments, while carbon dioxide solutions appeared to affect the eggs in general like an acid, yet they produced larvæ in Strongylocentrotus and not in Arbacia; while the reverse was true of hydrochloric acid. I consider the experiments too few, however, to be sure that this difference would always be found.

For reasons given later in the paper, I was led to try potassium cyanide dissolved in sea-water. In four experiments with Strongylocentrotus parthenogenetic larvæ were obtained, and some of them became plutei. Solutions and exposures which were successful were Experiment A, $\frac{m}{100}$, twenty-four hours and $\frac{m}{1000}$, forty-eight hours; Experiment B, $\frac{m}{200}$ and $\frac{m}{400}$, twenty-four hours; Experiment C, $\frac{m}{50}$, forty-two hours; Experiment D, $\frac{m}{50}$, forty-two hours. Not many larvæ were obtained, but a very great tendency to segmentation was noted. If eggs were left in the potassium cyanide solutions, nuclear and cell divisions began sooner or later, even sometimes in solutions as strong as $\frac{m}{50}$. This is remarkable when compared with my[1] re-

[1] LYON: This journal, 1902, vii, p. 56.

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sults on Arbacia at Woods Hole. In one experiment I find a record of many fine plutei produced in $\frac{m}{8000}$ and $\frac{m}{20000}$ potassium cyanide, the eggs having remained in the solutions continuously. Unfortunately no record of the control is found among my notes in this case.

3. **Sphærechinus granularis.** — This large species was brought in once only, and but one ripe female was found. Eggs treated with hydrochloric acid and potassium chloride in the same manner as Arbacia showed a great deal of segmentation, and a few good larvæ were found. The best result was from an exposure of two hours to a solution of 10 c.c. of $2\frac{1}{2}\,m$ potassium chloride in 100 c.c. of sea-water. Shaking gave no larvæ, although a large amount of segmentation was noted.

4. **Holothurians.** — Neither Holothuria tubulosa nor Holothuria stellata was ripe. The eggs would not fertilize, although the sperm used appeared very active. Eggs of the former were treated with hydrochloric acid, and a few pathological swimming larvæ were found. The successful solution contained 3 c.c. $\frac{m}{10}$ hydrochloric acid in 100 c.c. of sea-water, and it acted on the eggs five minutes. This solution and others caused a considerable amount of cell division, while the control was unchanged.

## Experiments on Ascidians.

**Ciona intestinalis.** — Although these animals are dioecious, Castle has shown that the eggs are practically always cross-fertilized. I confirmed this observation. Moreover, if one carefully cleaned the body wall with alcohol, and then punctured the oviduct, the eggs thus obtained from several individuals could be mixed without getting any development. As a rule, however, I kept the eggs from different individuals separate. Hydrochloric acid, potassium chloride, and carbon dioxide, in all strengths and exposures, gave no results. No tendency to cleavage, even, was noticed, although I made over forty experiments, and used several different lots of material. Mechanical agitation also failed.

## Relation of Oxygen to Artificial Parthenogenesis.

The great amount of cleavage that eventually took place in dishes of unfertilized but untreated eggs of Arbacia pustulata attracted attention early in my experiments. In Strongylocentrotus the ten-

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dency to division is hardly less marked. By studying the conditions under which this phenomenon took place, it seemed possible to gain some insight into the causes of artificial parthenogenesis. Thinking that the falling apart of the blastomeres which was so noticeable might be due to lack of oxygen, like the disintegration observed in my potassium cyanide experiments at Woods Hole, I tried the effect of pure oxygen. In two sterile flat-bottomed flasks of 250 c.c. capacity were placed equal quantities of sea-water and unfertilized eggs of Arbacia. The layers of liquid were less than one centimetre deep. Through one of the flasks a strong stream of well-washed and purified oxygen was passed for some minutes, and thereafter a slow stream. The gas did not pass through nor mechanically agitate the liquid. The other flask was covered with a small inverted beaker, and therefore exposed freely to the air. At the end of twenty-four hours, and at intervals thereafter, eggs were removed from each flask and examined.

The result was the reverse from what I had expected: division invariably began much sooner and went on to a much greater extent in the air-filled flask than in the oxygen-filled flask. The eggs in the oxygen remained in good condition and capable of fertilization after those in air had gone to pieces. I did not attempt to find limits; but in one experiment, for example, eggs kept in oxygen were fertilized after ninety hours and produced plutei, whereas those kept in air had all fallen into formless debris in less than sixty hours. While Arbacia unfertilized eggs in air showed a large percentage of cleavage in from twenty to twenty-four hours, those in pure oxygen often failed to show division in one egg in a thousand at the end of three days. These experiments succeeded equally well with Strongylocentrotus.

It would be impossible, or at least very difficult, to get the eggs of sea-urchins (especially Strongylocentrotus) wholly free from bacterial infection. Sooner or later all my cultures became putrid. This always happened earlier in the air-filled than in the oxygen-filled flask. One might, therefore, be inclined to attribute the earlier death of the eggs in air to bacteria. The truth is, rather, that the earlier presence of large numbers of bacteria in the air-filled flask was due to the earlier death of the eggs. Not until some time after the eggs had begun to fall apart in large numbers could bacteria be detected. The dead eggs furnished a good culture medium for the few germs present in the original preparation.

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It appeared, therefore, that lack of oxygen tended to induce division and development in sea-urchin eggs. It was this idea that led me to attempt to produce parthenogenesis by the use of potassium cyanide, as described earlier in this paper. The reasonable degree of success which attended these experiments pointed in the same direction. But whether a wider application of the method is possible, and whether all the means of producing artificial parthenogenesis really affect the respiration of the cells and produce a condition of lack of oxygen are matters of merely interesting conjecture at the present time.

Loeb[1] noticed that fertilized eggs of Fundulus, Ctenolabrus, and Arbacia in a current of hydrogen might segment a little before the control in air. I[2] found a slight hastening in the development of fertilized Arbacia eggs in very weak potassium cyanide solutions. Mathews[3] was able to start the development of Arbacia eggs by alternate exposures to hydrogen and air. These observations, together with those of Delage with carbon dioxide, and the action of anæsthetics observed by others, may eventually find a single explanation. Delage, however, expresses the belief that carbon dioxide (as also other agents which cause artificial parthenogenesis) does not act by causing asphyxia, but by arresting protoplasmic activity in some other way.

## Relation of Temperature to Parthenogenesis.

Loeb and others have noticed that temperature is an important factor in artificial parthenogenesis. A variation of a few degrees seems to make a great difference in the result. Few exact experiments, however, have been made. It is imaginable (1) that the temperature of the reagent to which the eggs are exposed is the important factor; or (2) that the temperature of the sea-water to which they are returned and left to develop is the deciding element. The few experiments which I made seemed to indicate that the temperature of the solution is of paramount importance. The temperature of the sea-water in which development takes place is also important, but chiefly so (between 16° and 22°) in affecting the rate of development. The experiments were too few, however, to

[1] J. Loeb: Archiv für die gesammte Physiologie, 1893, lv, p. 530.
[2] Lyon: This journal, 1902, vii, p. 56.
[3] Mathews: This journal, 1900, iv, p. 343.

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permit of more than tentative conclusions. But it seems to me the experiments speak against Delage's idea that the action of the solutions is inhibitory; that the essential factor in producing artificial parthenogenesis is a temporary suspension of activity. Some change is going on in the eggs during the time the reagent acts. The change is of such a nature that cell division can go on easier and faster afterwards. The change is slowed by lowering the temperature. That an active process is concerned is also indicated by such experiments as those I have described in Arbacia, where the time varied inversely as the strength of reagent used; *e.g.*, hydrochloric acid. Other investigators have announced similar observations.

I will describe some experiments on temperature: —

*Experiment 119.* — Three dishes were prepared, each containing 100 c.c. of sea-water + 14 c.c. of 2½ *m* KCl solution. These dishes were kept respectively at 17°, 20°, and 24° C. The mixed eggs of three specimens of Strongylocentrotus were distributed to these dishes. Eggs were removed after exposures of ½, 1, and 2¼ hours, and placed in sea-water at 20°. The half-hour exposure gave no results. The one hour exposure at 24° gave a very few larvæ; at 17° and 20°, no results. The 2¼ hour exposure at 17° and 20° gave numerous plutei; at 24°, one fragmentary blastula only.

*Experiment 119 a.* — Same as preceding, except that eggs after exposure to the solution were kept in sea-water at 17°. The exposures at 24° gave no results. Exposures at 17° and 20° for 2¼ hours gave numerous larvæ, which developed more slowly than those similarly treated in Experiment 119. The plutei formed were, perhaps, less uniformly normal. But some of them lived 18 days.

*Experiment 123.* — Two dishes for each of the following concentrations: — 100 c.c. sea-water + respectively 12 c.c., 14 c.c., 16 c.c. of 2½ *m* KCl. One dish of each concentration at 19°; the other at 23°. Eggs of Strongylocentrotus exposed 1, 1¼, 1½, 1¾, 2 hours, then placed in sea-water at room temperature, about 19°. Exposures of 1½ and 1¾ hours to the 14 c.c. and 16 c.c. solutions at both temperatures gave abundant larvæ. But 23° was much more favorable. Some of these formed plutei and lived 22 days. The 12 c.c. solutions gave no larvæ.

*Experiment 131.* Three dishes for each concentration: 100 c.c. sea-water + respectively 14 and 16 c.c. 2½ *m* KCl. One dish of each concentration kept at following temperatures, respectively, 15°, 19°, 23°. Eggs of Strongylocentrotus exposed 1⅓, 1½, 1¾, 2, and 4 hours. Removed to

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sea-water at room temperature, approximately 19°. Two hours after removal from the solutions one third to one half of those eggs which had been at 23° in either concentration for 1½ to 2 hours were in cleavage. Often as many as ten or twelve cells had been formed. In many eggs several nuclei were visible, although division of the cytoplasm had not taken place. Some of those exposed at 23° for four hours divided within forty minutes into ten or twelve cells. But none of the four hour specimens formed larvæ. The eggs treated at 19° and 15° were far behind the 23° eggs in division. By far the best and most numerous larvæ were from those eggs exposed at 23° for 1⅓ or 1½ hours to the solution of 16 c.c. 2½ *m* KCl in 100 c.c. sea-water.

The temperatures used by Greeley[1] and myself were so different that comparison of our results is of little value. My experiments do not uniformly bear out his conclusion that a long exposure at a low temperature gives results equal to, or better than, a shorter exposure at a higher temperature. But the temperatures used by me were not far apart, and the optimum time element introduces a factor of uncertainty. Unless the eggs are given, at each temperature, the length of exposure best adapted to it, comparative results are of no value. And the observer cannot be sure he has found the optimum time unless eggs were taken out at very frequent intervals. I did not always do this; and some of my experiments entirely support Greeley's view. I am therefore undecided as regards Strongylocentrotus, although before I saw Greeley's paper I was inclined to think 23° more favorable than 19° or 16°, irrespective of time.

## Summary.

1. The eggs of the sea-urchins at Naples may be caused to develop (during September, October, November, at least) by approximately the same means used by Loeb at Woods Hole.

2. In a few instances larvæ of Strongylocentrotus were obtained by the use of carbon dioxide.

3. In four experiments larvæ of Strongylocentrotus were obtained by the use of potassium cyanide.

4. The pronounced tendency of Arbacia pustulata and Strongylocentrotus lividus unfertilized eggs to segment after about twenty to twenty-four hours can be overcome by exposing the eggs to pure oxygen.

[1] Greeley: Biological bulletin, 1903, iv, p. 129.

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5. The temperature of the solution to which the eggs are exposed to induce parthenogenesis is very important. The temperature of the sea-water to which the eggs are returned for development is probably less vital, although the rate is slower at low temperatures.

6. All efforts to cause the parthenogenetic development of Ciona intestinalis failed.

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# THE RELATIONSHIP BETWEEN THE FREEZING POINT DEPRESSION AND SPECIFIC GRAVITY OF URINE, UNDER VARYING CONDITIONS OF METABOLISM, AND ITS CLINICAL VALUE IN THE ESTIMATION OF SUGAR AND ALBUMIN.[1]

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## Introduction.

In the course of his work on the theory of dilute solutions Van't Hoff developed the relationship existing between their chemical constitution, osmotic pressure, and freezing point. Raoult subsequently made use of the freezing point of solutions as a direct means of determining the molecular weight of the dissolved substance, and it is to this investigator and Beckmann that we are indebted for the simple apparatus now universally employed for this purpose. For many years Beckmann's apparatus has been in constant use in most physiological chemical laboratories for determining the freezing point of

[1] This paper was read before the Buffalo and Niagara Falls Chemical Society in March, 1903.



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blood, urine, etc. Unfortunately these determinations have usually been made with a purely practical object in view, the comparison of osmotic pressures, very little attention being paid either to Van't Hoff's theoretical generalization of Avogadro's law upon which the method itself is really based, or to the valuable deductions which may be drawn by this means regarding the nature and proportions of the individual substances producing the osmotic effect.

Van't Hoff's and Raoult's conclusions may be expressed as follows:

1. The osmotic pressure exerted by any substance in solution is independent of the nature of the molecules of that substance and simply proportional to their number. And this pressure corresponds with that which would be exerted by an equal number of molecules of any ideal gas under the same physical conditions.

2. The lowering of the freezing point of any solution below that of the solvent employed is directly proportional to the osmotic pressure and consequently to the number of molecules dissolved, regardless of their nature. Or, expressed in another form, solutions of different substances in the same solvent, dissolved in such proportions as to have the same freezing point, are isosmotic and equimolecular.

It follows from these laws that the average size of the molecule or dissociated ion present in a given physiological solution may be estimated as directly proportional to the weight of substance dissolved in a given quantity of fluid, and inversely proportional to the lowering of the freezing point (or osmotic pressure) of the solution. This fact has been expressed by Raoult and Beckmann in the form of a simple equation;

$$M = E\frac{m}{\Delta},$$

where $M$ is molecular weight of substance in question, to be determined; $E$ is a constant having the value of 18.5, when water is employed as the solvent; $m$ is the known weight of substance dissolved in 100 c.c. of solvent, and $\Delta$ is the observed depression of the freezing point in degrees C.[1]

Hoping by this method to throw some light upon the nature of the disturbances in metabolism taking place throughout the body in the

[1] When, as in the case of urine, the exact weight of dry solids cannot be accurately determined without considerable loss of time, an approximate value of $m$ may very readily be obtained by multiplying the specific gravity of the solution, taken at 15° C., by the factor 2.33; thus for $m$ we may substitute specific gravity multiplied by 2.33.

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course of such diseases as cancer, diabetes, etc., we have made a practice of determining the freezing point of stomach contents, blood, transudates and urines, both as a whole, and also in separate fractions, in those cases in which allowance could be made for the influence exerted by the fractionating agents employed. The specific gravity as well as the proportions of the various chemical constituents of the fluid in question were determined, as a matter of routine, in the course of our experiments on metabolism; and the relationship obtaining between these quantities and the freezing point was calculated. This paper will be especially devoted to the consideration of certain relationships existing between the freezing points and specific gravities of normal urine; and further, to deviations from the normal observed in diabetic urines, which afford a simple means of estimating the sugar which they contain.

## Specific Gravity and Depression of Freezing Point of Normal Urines.

The lowering of the freezing point in the normal urine varies within very wide limits, being directly influenced by the consumption of water in the same manner as is the specific gravity. In fact, on comparing the freezing point directly with the specific gravity, a constant relationship is found to obtain which only varies within such narrow limits as almost to fall within the range of experimental error.[1]

This will be seen from an examination of Table I, representing a series of experiments made from day to day on the writer's urine, and Table II, a series of determinations on the urines of normal individuals, the only regulations observed being a full normal diet and restriction within reasonable limits in the consumption of various salts, medicaments, and alcoholic stimulants.

[1] This same observation has been made by Fuchs, and the result published in the Zeitschrift für angewandte Chemie, in October, 1902, an abstract of his article appearing in the Biochemisches Centralblatt, January, 1903. Were it not that his theoretical conclusions are open to criticism, and that those phases of his work bearing directly upon the estimation of sugar in diabetic urines leave much to be desired, the publication of these results at this early date would be unnecessary. Realizing that the empirical factors published by Fuchs needed revision, lest they bring an excellent method of analysis into disrepute, we felt that the immediate publication of this portion of the work (apart from the complete studies in metabolism referred to above) would be justified.

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In these tables the first column represents the specific gravity of urine determined at 15° C.; the second column gives the depression of the freezing point of the urine in question below that of water, and is indicated by $\Delta$; the third column, represented by $\Delta'$, gives the calculated freezing point derived from the decimal portion of the specific gravity by means of the factor 75, the source of which will be explained later; the fourth column shows the relationship existing between *S*, the decimal portion of the specific gravity determined at 15° C., and $\Delta$, the observed freezing point depression. An inspection of these

TABLE I.

| Day. | Spec. grav. at 15° C. | F. P. Observed $\Delta$. | F. P. Calculated $\Delta'$. | $\frac{S}{\Delta}$. | $\frac{\Delta}{S}$. |
|---|---|---|---|---|---|
| 1 | 1.0252 | 1.87 | 1.89 | 0.0134 | 74 |
| 2 | 1.0135 | 1.02 | 1.01 | 0.0132 | 75 |
| 3 | 1.0195 | 1.485 | 1.46 | 0.0131 | 76 |
| 4 | 1.0230 | 1.67 | 1.72 | 0.0137 | 73 |
| 5 | 1.0185 | 1.38 | 1.39 | 0.0134 | 74 |
| 6 | 1.0237 | 1.79 | 1.78 | 0.0132 | 75 |
| Average . . . . . . . | | | | 0.0133 | 75 |

figures makes it appear evident that $\frac{S}{\Delta}$ = a constant, about 0.01337. In both cases the average of a large number of determinations is found to be about the same, and the variations are, in no case, more than 4 per cent from the mean. Expressed in another form, each 0.01 of specific gravity is found to be approximately equivalent to a lowering of the freezing point of $\frac{1}{1.337} = 0.75°$ C. It will now be seen from what source the factor 75, made use of in the third column and referred to above, is derived. Each 0.01 of the decimal portion of the specific gravity of a normal urine is equivalent to 0.75° C. lowering of the freezing point, and the theoretical freezing point of any urine may be determined by multiplying the decimal portion of the specific gravity by the factor 75. For example, in the first

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case on Table I, the specific gravity = 1.0252; then 0.0252 × 75 = 1.89, a figure which compares very closely with the observed freezing point 1.87. In a second case, taken from Table II, No. 9, specific gravity is 1.0202, the observed freezing point 1.51°; 0.0202 × 75 = 1.51, which exactly coincides with the observed freezing point. These

TABLE II. NORMAL INDIVIDUALS.

| Case. | Spec. grav. at 15° C. | F. P. Observed $\Delta$. | F. P. Calculated $\Delta'$. | $\frac{S}{\Delta}$. | $\frac{\Delta}{S}$. |
|---|---|---|---|---|---|
| 1 | 1.0126 | 0.98 | 0.94 | 0.0128 | 78 |
| 2 | 1.0114 | 0.885 | 0.86 | 0.0129 | 78 |
| 3 | 1.0287 | 2.19 | 2.15 | 0.0131 | 76 |
| 4 | 1.0255 | 1.96 | 1.91 | 0.0130 | 77 |
| 5 | 1.0248 | 1.88 | 1.86 | 0.0132 | 76 |
| 6 | 1.0112 | 0.85 | 0.84 | 0.0132 | 76 |
| 7 | 1.0132 | 0.98 | 0.99 | 0.0135 | 74 |
| 8 | 1.0242 | 1.815 | 1.815 | 0.0133 | 75 |
| 9 | 1.0202 | 1 51 | 1.51 | 0.0134 | 75 |
| 10 | 1.026 | 1.94 | 1.95 | 0.0134 | 75 |
| 11 | 1.0259 | 1.915 | 1.94 | 0.0135 | 74 |
| 12 | 1.0255 | 1.86 | 1.91 | 0.0137 | 73 |
| 13 | 1.0225 | 1.63 | 1.69 | 0.0138 | 72 |
| 14 | 1.0225 | 1.63 | 1.69 | 0.0138 | 72 |
| 15 | 1.0219 | 1.56 | 1.64 | 0.0140 | 71 |
| | | Average . . . . . . . | | 0 01337 | 75 |

same factors are found to hold good, although within slightly wider limits, in the case of normal individuals under abnormal conditions, and in the urine of patients suffering from various diseases and undergoing varying treatments, provided sugar and albumin are absent. This phase of the subject will be dealt with in the next section.

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## Specific Gravity and Depression of Freezing Point of the Urine of Normal Individuals under Abnormal Circumstances, and of Pathological Urines Other than Those Containing Sugar and Albumin.

It is not proposed at this stage to enter into a minute study of the chemical constitution of various urines as compared with their freezing point and its deviation from the theoretical. That phase of the subject will be discussed in a later section. It is, however, interesting to note that the increase in the proportion of chlorides and other salts having smaller ions than the average in urine, produces less effect upon the freezing point than would be anticipated. It is even more remarkable that pathological urines, as, for example, those of cancer patients in the last stages, in which the proportion of normal constituents is seriously interfered with, and the chlorides have practically disappeared, should retain their normal freezing point within very narrow limits, as will be seen from an inspection of Tables III and IV, cancer cases.

The same close agreement was obtained in the urine of a typhoid fever case which for several days was almost free from chlorides and contained as much as 3 per cent of urea.

TABLE III. CANCER CASE H.

| Day. | Spec. grav. at 15° C. | F. P. Observed $\Delta$. | F. P. Calculated $\Delta'$. | $\frac{S}{\Delta}$. |
|---|---|---|---|---|
| 1 | 1.0152 | 1.162 | 1 14 | 0.0130 |
| 2 | 1.0140 | 1.105 | 1.05 | 0.0126 |
| 3 | 1.0125 | 0.930 | 0.940 | 0.0134 |
| | | Mean | . . . . . . . | 0 0130 |

Table IV gives results obtained during the last three weeks of his life, from the urine of a patient suffering from cancer of the stomach. This urine contained not more than one-tenth to one-twentieth of the normal proportion of chlorides, and yet, as will be seen, the average value of $\frac{S}{\Delta}$ does not vary to any considerable extent from the normal.

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TABLE IV. CANCER CASE W.

| Day. | Spec. grav. at 15° C. | F. P. Observed $\Delta$. | F. P. Calculated $\Delta'$. | $\frac{S}{\Delta}$. | $\frac{\Delta}{S}$. |
|---|---|---|---|---|---|
| 1 | 1.020 | 1.46 | 1.50 | 0.0137 | 73 |
| 2 | 1.0198 | 1.38 | 1.49 | 0.0143 | 70 |
| 3 | 1.019 | 1.41 | 1.42 | 0.0134 | 74 |
| 4 | 1.0125 | 0.92 | 0.94 | 0.0136 | 74 |
| 5 | 1.0162 | 1.19 | 1.21 | 0.0136 | 73 |
| 6 | 1.0135 | 1.00 | 1.01 | 0.0135 | 76 |
| 7 | 1.0152 | 1.07 | 1.14 | 0.0142 | 70 |
| 8 | 1.0121 | 0.91 | 0.91 | 0.0133 | 75 |
| 9 | 1.0117 | 0.92 | 0.88 | 0.0127 | 78 |
| 10 | 1.0124 | 0.87 | 0.93 | 0.0142 | 70 |
| 11 | 1.0141 | 1.04 | 1.06 | 0.0135 | 74 |
| 12 | 1.0103 | 0.79 | 0.77 | 0.0130 | 76 |
| | | Mean . . . . . . . | | 0.0136 | 74 |

The urine of patients before and after epileptic seizure has also been examined in a considerable number of cases. The freezing point has been found to remain fairly constant within reasonable limits. We have also included the urine of animals in our series of experiments. Table V gives the results obtained in two cases from a series of four guinea-pigs, from which it will be seen that a fair uniformity exists amongst the individual animals, although the difficulties associated with the accurate determination of the specific gravity lead to a much larger experimental error in the case of animals' urine, where the normal specific gravity is from one-third to one-half that of human beings.

It will be seen from a consideration of the results enumerated in this section, that a fairly constant relationship is found to obtain between the specific gravity and freezing point, even under the most abnormal conditions, and in urines of widely varying chemical composition. On the other hand, no constant proportion can be traced between the chlorides, or any other chemical constituent either of

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normal or pathological urines, and the freezing point. Merely the sum total of effects is a constant, the individual components varying

TABLE V. URINE OF EXPERIMENTAL GUINEA-PIGS.

| Pig. | Spec. grav. (approx.) at 15° C. | F. P. Observed $\Delta$. | F. P. Calculated $\Delta'$. | Difference. | $\frac{\Delta}{S}$. |
|---|---|---|---|---|---|
| 1 | 1.009 | 0.61 | 0.67 | 0.06 | 68 |
| 2 | 1.012 | 0.86 | 0.90 | 0.04 | 72 |
| 3 | 1.010 | 0.71 | 0.75 | 0.04 | 71 |
| 4 | 1.011 | 0.73 | 0.82 | 0.09 | 66 |
| 1 | 1.010 | 0.72 | 0.75 | 0.03 | 72 |
| 2 | 1.011 | 0.83 | 0.83 | nil | 75 |
| 3 | 1.011 | 0.80 | 0.83 | 0.03 | 73 |
| 4 | 1.0105 | 0.72 | 0.79 | 0.07 | 69 |

within wide limits. The actual influence exerted by various constituents of the urine, and the way in which the proportions of these substances are adjusted, will be considered briefly at a later stage.

## The Average Molecular or Ion Weight of the Substances Dissolved in Urine.

From the observation referred to above, that in normal urine the depression of the freezing point is about 0.75° for each 0.01 in the decimal of the specific gravity, it is comparatively simple to calculate the average molecular weight of the individual ions present in urine from the formula referred to above;

$$M = 18.5 \frac{m}{\Delta},$$

if we assume that each 0.01 in the specific gravity determined at 15° is equivalent to 2.33 per cent of normal solids in the urine.[1] Then,

[1] A large number of determinations of the dry solids in urine have been made by the writer from time to time, which have proved very conclusively that the factor 2.3 to 2.4, as commonly accepted, does not always represent the correct figure, but merely an average. Since, however, work of the nature described above

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$$M = 18.5 \frac{2.33}{0.75} = 57.5.$$

We have several times in the course of our work compared the average molecular weight as obtained from the specific gravity, with that obtained from the total weight of solids found on evaporating the urine. The average of a large number of determinations is practically the same, but the figures obtained, making use of the specific gravity, are much more uniform than those obtained from the weight of solids direct. In fact, it may be said that the factor $\frac{\text{sp. gr.}}{\text{F.P.}}$ is much more nearly constant than is the factor $\frac{\text{total solids}}{\text{F. P.}}$. There are two possible explanations for this: the first is the difficulty of determining total solids correctly, and the second is the fact that urea and salt (NaCl), the two most important constituents of the urine (so far as specific gravity, total solids, and freezing point are concerned), whilst possessing ions differing widely in their relative average weights of 60 and 30, also exert a very different effect upon the specific gravity, but in the inverse direction, such that aqueous solutions of urea or sodium chloride possessing the same specific gravity contain more nearly equal quantities of ions, than do solutions of these substances of equal percentage concentration. The most important factors in determining approximately the specific gravity and freezing point of urine, are: (1) the percentage of urea, chlorides, and other readily dissociated salts producing a very marked effect upon the freezing point, and (2) the percentage of those residual organic constituents of the urine, such as uric acid, creatinine, pigments, and other organic substances which may be looked upon as practically inert so far as effect upon the osmotic pressure or freezing point is concerned.

Since such a constant relationship appears to obtain between the specific gravity and freezing point, it must be assumed that a constant equilibrium is maintained between these two types of urinary constituents. Since the average molecular weight is slightly less

is not absolute, but merely relative; and since the principal endeavor is to reach an average, and that within a fairly wide range of experimental error, it has been decided to use the factor 2.33 when dealing with normal urines. As will be shown later, it is necessary to employ 2.8 as the factor for sugar solutions, and 3.8 to 4 for those of albumin, so far as these individual constituents are concerned. It will also be seen that in the estimation of sugar by this method referred to later, it is a matter of indifference whether we employ the factor 2.33, 2.2, or even 2.0.

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than 60, and the average of the electrolytes lies considerably below that figure, it must be assumed that some direct relationship exists between the proportion of these bodies and of the non-conducting, large molecular complexes present in the urine. This question will be dealt with in the next section.

Disregarding the total weight of solids and specific gravity, we have in several cases calculated the probable average size of the ion in a series of normal urines, allowing for the effect exerted by each individual constituent at the concentration in question, and have invariably obtained figures lying between 50 and 60, usually about 55. It will be impossible to present the data bearing on this question at present, as the material involved would carry us too far from the main object of this paper, the analysis of sugar urines, etc., dealt with in a later section.

## The Influence Exerted by Individual Ions.

As stated in the last section, the first important factors to determine are: (1), What proportion of the total nitrogen of the urine is present in the form of urea; (2) The variation in the proportion of chlorides from the normal, and (3) The amount of inert or non-dissociated material. The variations in the amount of chlorides in urine is very marked. In certain normal cases, the total excretion of sodium chloride may reach a figure equal to that of urea. In the last stages of cancer, in typhoid fever, etc., when the proportion of chlorides in the diet is considerably restricted, and in addition the body shows a tendency to retain those salts, the excretion of chlorides may fall to one-twentieth part of the normal or even entirely disappear. Such variations in the proportion of those bodies having the smallest ionic weight should, if other constituents remain normal, lead to a very marked variation of the relationship between specific gravity and freezing point. We must, therefore, assume that some condition of equilibrium actually exists within definite limits between the proportion of salts possessed of small ions and organic bodies possessed of a large molecular complex. It is true that in such pathological conditions the amount of ammonia in proportion to the total nitrogen is increased two and three times the normal, but the total quantity is, in any case, too small to account for more than a small portion of the effect produced by a diminution in chlorides; and, in such a case as that dealt with in Table IV, the ammonia would be practically

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counterbalanced by the uric acid increase. The phosphates and sulphates, whilst varying within fairly wide limits, possess, on the average, larger ions than the chlorides, and do not, under normal circumstances, constitute nearly so large a proportion of the total. The influence which they exert, after allowing for their organic combinations, does not vary to a very considerable extent.

It is not our intention to deal with this question from a theoretical standpoint in this paper, as any ideas advanced in this direction must necessarily be purely speculative; but we consider it highly probable that there is a tendency toward the maintenance of a definite state of equilibrium between the various organic excretory products and the simple salts, such as chlorides, sulphates, etc., which may account for the non-removal from the body, in the course of certain diseases, of the products of sub-oxidation and impaired metabolism when an insufficient supply of salts is excreted with the urine. These bodies accumulate in the system, and may, in their turn, retain, associated with themselves, those quantities of salts which are introduced with the diet. This phase of the subject, whilst of considerable importance, cannot be dealt with in this paper.

## Therapeutic Treatment with Chlorides.

Considerations of this nature may, however, be looked upon as a further argument in favor of the addition of sodium chloride to the milk diet of patients suffering from fevers, cancer, etc., a procedure frequently resorted to by clinicians, as the result of practical experience. This course is advocated by Hatcher and Sollman,[1] in a paper dealing very completely with the effect of diminished sodium chloride excretion in typhoid fever. Unfortunately, these authors have not recorded the specific gravity of the urine in their tables, and since they themselves consider the figures referring to total solids as inaccurate, the factor, $\frac{\Delta}{\text{sp. gr.}}$ or $\frac{\Delta}{\text{total solids}}$, derived from their work is very far from a constant. There is, however, an indication that the large excess of sodium chloride which they administered brought about a disturbance in equilibrium of the system in a direction opposite to that exhibited in the total absence of chlorides, and it appears probable that the best state of equilibrium was maintained when not more

[1] Hatcher and Sollman: This journal, 1902, vol. viii, page 139.

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than five or six grams of sodium chloride were excreted in the space of twenty-four hours. We must, however, leave this subject for a later paper dealing with disturbances in chloride equilibrium in general.

## Analytical Methods based upon the Fact that the Average Weight of the Ion in Urine is Slightly Less than 60.

In diabetic urine, for example, we have to deal with a solution containing an admixture of normal urinary constituents having an average molecular weight of about 60, and sugar molecules having an average weight of 180. The estimation of sugar in diabetic urine, based upon this relationship, will be dealt with at a later stage. The estimation of albumin present in solution in urine, dependent upon the fact that the molecule of albumin is so large as to produce a practically negligible effect upon the freezing point, will be treated in Section 11.

**Estimation of sugar in diabetic urines.** — Since the average molecular weight of the normal substances present in urine is 57.5, and that of sugar is 180, it is obvious from a consideration of Van't Hoff's generalization referred to at the commencement of this paper, that the effect produced upon the osmotic pressure of any solution by a given weight of sugar would be slightly less than one-third (that is to say, in the proportion of $\frac{57.5}{180}$) of that exerted by an equal weight of normal urinary solids. Now, if the effect exerted upon the specific gravity of solutions due to the introduction of a given weight of sugar be the same as that due to the solution of the same weight of normal urine constituents, the problem involved would be comparatively simple; but this is not the case.

As the result of a series of careful determinations made in the Laboratory, we find that the proportions are as six to five; that is to say, six grams of sugar dissolved in one hundred of water would produce a solution having about the same specific gravity as five grams of ordinary urine solids in one hundred of water. Consequently the factor 2.33 cannot be applied to sugar-containing urines in order to determine the weight of substance dissolved. We have to make use of another factor for the sugar, which is about 2.81. (See note, page 326.)

Before making the first freezing point determination on diabetic urine, the following calculation was carried out. If the freezing point

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of the diabetic urine were first determined, and then the theoretical freezing point of the same urine were deduced from the specific gravity, making use of the factor 75, as though it were normal urine, the difference between these two freezing points would be due to the relatively larger size of the sugar molecules as compared to the normal urine molecules. Whilst exerting slightly less effect upon the specific gravity than would an equal weight of urine solids, the sugar should merely exert one-third of the effect upon the freezing point that would normally be expected from urine constituents.

If the specific gravity were influenced to the same extent by sugar and normal urine solids, since the molecule of the latter is about one-third of the sugar molecule, each sugar molecule would be represented in the freezing point on the normal urine basis, only to the extent of one-third of its weight, the remaining two-thirds being accounted for by the difference between the observed and theoretical freezing point, and one-half of that figure would consequently be equivalent to the number of sugar molecules present in the solution; from which it will be seen that it would only be necessary to multiply the difference between the theoretical and observed freezing points of such urine by the factor 5, in order to determine the percentage of sugar in the solution.

The effect exerted by sugar and normal urine solids upon the specific gravity is, however, not the same. As stated above, five grams of the normal solids produce about the same result as six grams of sugar, so that if the normal factors made use of in calculating the solids of urine be employed, the molecule of sugar would appear as merely $\frac{5}{6}$ of $180 = 150$. Since this 150 functions in the same manner as $57\frac{1}{2}$ of the normal urine constituents, the difference, $150 - 57\frac{1}{2} = 92\frac{1}{2}$, is unaccounted for and is represented by the difference between the theoretical and observed freezing point; and in order to determine the percentage of sugar we should multiply the difference in question by the factor $\frac{57\frac{1}{2}}{92\frac{1}{2}}$, which is about 0.6. A simple mathematical derivation of the factor 6 employed in our work is included in the form of a footnote.[1]

[1] 0.75° of lowering of the F. P. is equivalent to 0.01 of specific gravity of urine when the latter is induced by normal solid constituents of urine without the admixture of sugar or albumin. It may be calculated and also shown by experiment, that 0.75° is equivalent to 0.026 of specific gravity due to sugar in any dextrose-containing solution; consequently if we represent the decimal portion of the

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We have since made a large number of determinations of the sugar in urine by this method, multiplying the difference between the theoretical and observed freezing points by the factor 6, and have compared results thus obtained with the analyses by means of polarization of light, fermentation of sugar, loss of specific gravity due to fermentation, reduction of Pavy's solution, etc. This method has shown itself extremely accurate in almost all cases and no special precautions have been taken at any time. It has been our endeavor, so far as possible, to carry on analyses under those conditions which obtain in the ordinary clinical laboratory, so that it may not be said that this method is one which can only be made use of where special physico-chemical corrections for errors may be introduced.

The following tables, VI, VII, and VIII, refer to the urine of patients suffering from diabetes; the first column giving the specific gravity, the second the observed, and the third the calculated freezing point; the fourth gives the difference between these latter, and the fifth the percentage of sugar calculated from the above by means of the factor 6; the sixth column gives the determination of the sugar by polarization of light, and the seventh any check determination that may have been made by some other dependable method; the eighth gives the value of $\frac{S}{\Delta}$, which varies considerably from the normal (0.0133), in these cases.

specific gravity of the urine as $S$ and the fraction of that specific gravity due to the action of sugar as $x$, then,

1. $$\frac{S}{0.01} \times 0.75^\circ = \text{theoretical F. P.}$$

2. $$\frac{S}{0.026} \times 0.75^\circ = \text{theoretical F. P. of a pure sugar solution.}$$

3. $$\left(\frac{Sx}{0.026} + \frac{S(1-x)}{0.01}\right) 0.75^\circ = \text{the observed F. P.}$$

Subtracting equation 3 from equation 1,

$$Sx = \frac{26\ (\text{Diff. in F. P.})}{1200};$$

and since the weight of sugar in 100 c.c. = the portion of the specific gravity due to sugar, multiplied by 280,

$$\text{Weight of sugar \%} = \frac{26 \times 280\ (\text{Diff. in F. P.})}{1200} = 6 \text{ times Diff. in F. P.}$$

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TABLE VI. DIABETES CASE A.

| Day. | Sp. gr. at 15° C. | F. P. Observed. | F. P. Calculated. | Diff. | Sugar diff. × 6. | Sugar by pol'n. | Sugar loss in sp. gr. | $\frac{S}{\Delta}$. |
|---|---|---|---|---|---|---|---|---|
| | | | | | per cent. | per cent. | per cent. | |
| 1 | 1.0349 | 1.50 | 2.62 | 1.12 | 6.7 | 6.8 | .. | 0.023 |
| 2 | 1.0334 | 1.42 | 2.50 | 1.08 | 6.5 | 6.3 | .. | 0.0235 |
| 3 | 1.0369 | 1.565 | 2.77 | 1.205 | 7.2 | 7.2 | .. | 0.0235 |
| 4 | 1.033 | 1.325 | 2.47 | 1.145 | 6.9 | 7.3 | 7.0 | 0.0249 |
| 5 | 1.0376 | 1.475 | 2.82 | 1.345 | 8.1 | 8.1 | 7.8 | 0.025 |
| 6 | 1.0388 | 1.86 | 2.91 | 1.05 | 6.3 | 6.4 | .. | 0.0208 |
| 7 | 1.0375 | 1.94 | 2.81 | 0.87 | 5.2 | 5.3 | 5.4 | 0.019 |
| 8 | 1.0351 | 1.90 | 2.63 | 0.73 | 4.4 | 4.2 | .. | 0.018 |
| 9 | 1.0332 | 1.835 | 2.49 | 0.655 | 3.9 | 3.8 | .. | 0.018 |
| 10 | 1.0327 | 1.865 | 2.45 | 0.585 | 3.5 | 3.5 | .. | 0.0175 |
| 11 | 1.033 | 1.845 | 2.475 | 0.63 | 3.8 | 3.8 | 4.0 | 0.0179 |
| 12 | 1.0332 | 1.72 | 2.49 | 0.77 | 4.6 | 4.5 | .. | 0.019 |
| 13 | 1.033 | 1.70 | 2.475 | 0.775 | 4.7 | 4.5 | .. | 0.02 |
| 14 | 1.0378 | 1.90 | 2.83 | 0.93 | 5.6 | 5.6 | .. | 0.0199 |
| 15 | 1.0341 | 1.89 | 2.56 | 0.67 | 4.0 | 4.0 | .. | 0.018 |
| 16 | 1.0365 | 1.70 | 2.74 | 1.04 | 6.2 | 6.2 | .. | 0.021 |
| 17 | 1.0376 | 1.82 | 2.82 | 1.00 | 6.0 | 6.0 | .. | 0.020 |

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TABLE VII. DIABETES CASE B.

| Day. | Sp. gr. at 15° C. | F. P. Observed. | F. P. Calculated. | Diff. | Sugar diff. × 6. | Sugar by pol'n. | Sugar loss in sp. gr. | $\frac{S}{\Delta}$. |
|---|---|---|---|---|---|---|---|---|
| | | | | | per cent. | per cent. | per cent. | |
| 1 | 1.0376 | 1.605 | 2.82 | 1.22 | 7 3 | 7.3 | 7.4 | 0.0234 |
| 2 | 1.0315 | 1.40 | 2.36 | 0.96 | 5.8 | 5.9 | .. | 0.0225 |
| 3 | 1.0265 | 1.37 | 1.99 | 0.62 | 3.7 | 3.5 | .. | 0.0193 |
| 4 | 1.0271 | 1.44 | 2.03 | 0.59 | 3.5 | 3.3 | 3.4 | 0.0188 |
| 5 | 1.0221 | 1.375 | 1.66 | 0.285 | 1.7 | 2.0 | .. | 0.0160 |
| 6 | 1.0285 | 1.63 | 2.14 | 0.51 | 3.0 | 2.9 | .. | 0.0174 |
| 7 | 1.0363 | 1.77 | 2.72 | 0.95 | 5.7 | 5.75 | .. | 0.0205 |
| 8 | 1.0372 | 1.83 | 2.79 | 0.96 | 5.75 | 5.7 | .. | 0.0203 |
| 9 | 1.0376 | 1.77 | 2.82 | 1.05 | 6.3 | 6.5 | .. | 0.0212 |

TABLE VIII. DIABETES CASE C.

| Day. | Sp. gr. at 15° C. | F. P. Observed. | F. P. Calculated. | Difference. | Sugar diff. × 6. | Sugar by pol'n. | Sugar loss in sp. gr. |
|---|---|---|---|---|---|---|---|
| | | | | | per cent. | per cent. | per cent. |
| 1 | 1.0275 | 1.29 | 2.06 | 0.77 | 4.6 | 5.0 | |
| 2 | 1.0247 | 1.40 | 1.85 | 0.45 | 2.7 | 2.7 | |
| 3 | 1.018 | 1.247 | 1.35 | 0.103 | 0.6 | 0.6 | |
| 4 | 1.0225 | 1.355 | 1.69 | 0.335 | 2.0 | 2.0 | |
| 5 | 1.0235 | 1.225 | 1.76 | 0.535 | 3.2 | 3.0 | 3.0 |
| 6 | 1.0165 | 1.089 | 1.24 | 0.15 | 0.9 | 0.6 | |
| 7 | 1.0138 | 0.963 | 1.035 | 0.072 | 0.43 | 0.5 | |
| 8 | 1.0126 | 0.965 | 0.945 | .... | nil | nil | |
| 9 | 1.0192 | 1.49 | 1.44 | .... | nil | nil | |
| 10 | 1.0204 | 1.205 | 1.53 | 0.325 | 1.95 | 1.9 | 1.8 |
| 11 | 1.0199 | 1.121 | 1.49 | 0.369 | 2.2 | 2.0 | 1.7 |
| 12 | 1.0168 | 1.26 | 1.26 | .... | .. | nil | nil |
| 13 | 1.0093 | 0.748 | 0.70 | .... | .. | nil | nil |
| 14 | 1.0166 | 1.055 | 1.245 | 0.19 | 1.1 | 1.0 | 1.1 |
| 15 | 1.019 | 1.055 | 1.425 | 0.37 | 2.2 | 2.0 | 2.1 |

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As an example of the method of calculation, taking the first determination in Table VI, the urine having a specific gravity of 1.0349 had a freezing point 1.50° below that of water.

The decimal portion of the specific gravity,

$$0.0349 \times 75 = 2.62°.$$

The difference between theoretical and observed freezing points,

$$2.62 - 1.50 = 1.12°,$$
$$1.12 \times 6 = 6.72 \text{ per cent sugar.}$$

The result obtained by polarization was 6.8 per cent. A second example, the first on Table VII, showed a specific gravity of 1.0376 and an observed freezing point of 1.605°.

The decimal portion of the specific gravity,

$$0.0376 \times 75 = 2.82,$$
$$2.82 - 1.605 = 1.225,$$
$$1.225 \times 6 = 7.35 \text{ per cent of sugar.}$$

In this case the polarization method gave 7.3 per cent, and the loss of specific gravity by fermentation 7.4 per cent.

**Estimation of sugar in artificially prepared sugar urines.** — In order to confirm these results, I prepared some artificial sugar urines, by

TABLE IX.

| Sugar added. | Spec. grav. at 15° C. | F. P. Observed. | F. P. Calculated. | Diff. | Sugar diff. × 6. | Sugar by rotation. |
|---|---|---|---|---|---|---|
| per cent. | | | | | per cent. | per cent. |
| 0 | 1.0239 | 1.79 | 1.79 | nil | nil | nil |
| | Diff., 0.0073 | Diff., 0.215 | | | | |
| 2.03 | 1.0312 | 2.005 | 2.34 | 0.335 | 2.01 | 2.0 |
| | Diff., 0.0073 | Diff., 0.205 | | | | |
| 4.06 | 1.0385 | 2.21 | 2.89 | 0.68 | 4.08 | 4.0 |

TABLE X.

| Sugar added. | Spec. grav. at 15° C. | F. P. Observed. | F. P. Calculated. | Diff. | Sugar diff. × 6. | Sugar by rotation. | Sugar by fermentat'n. |
|---|---|---|---|---|---|---|---|
| per cent. | | | | | per cent. | per cent. | per cent. |
| 0 | 1.0250 | 1.87 | 1.87 | nil | nil | nil | nil |
| | Diff., 0.0106 | Diff., 0.30 | | | | | |
| 3.0 | 1.0356 | 2.17 | 2.67 | 0.50 | 3.0 | 3.1 | 3.1 |
| | Diff., 0.0107 | Diff., 0.31 | | | | | |
| 6.0 | 1.0463 | 2.48 | 3.47 | 0.99 | 5.9 | 5.9 | 5.8 |

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dissolving varying proportions of sugar in normal urines of known specific gravity and freezing point. In Tables IX, X, and XI, three such series of experiments are given.

TABLE XI.

| Sugar added. | Spec. grav. at 15° C. | F. P. Ob-served. | F. P. Cal-culated. | Diff. | Sugar diff. × 6. | Sugar by rotation. |
|---|---|---|---|---|---|---|
| per cent. 0 | 1.0228 | 1.715 | 1.71 | nil | per cent. nil | per cent. nil |
| 2.65 | 1.0334 | 2.05 | 2.505 | 0.455 | 2.73 | |
| 4.90 | 1.0413 | 2.295 | 3.10 | 0.805 | 4.83 | 4.86 |

In order to facilitate the work and save unnecessary correction, those urines were chosen which possessed an exactly normal freezing point. The effect upon the specific gravity and freezing point on adding two and four grams respectively in the first series, and three and six grams in the second series, was carefully observed. It was found that the effect exerted upon the specific gravity by each 1 per cent of sugar added was about equal to 0.00355 to 0.0036, whilst the influence exerted upon the freezing point varied between 0.100 and 0.107, the theoretical being 0.103. Thus, the average effect upon the freezing point was so nearly equal to the theoretical that the molecular weight of sugar might be calculated by means of the formula

$$M = \frac{18.5\ m}{\Delta} = \frac{18.5}{103} = 180. \qquad \text{(See Table IX.)}$$

The difference between the observed and calculated freezing point for 2.03 grams of sugar added, equal 0.335°, which, multiplied by 6 = 2.01 per cent of sugar, the rotation showing 2 per cent. On addition of 4.06 grams, the effect upon the freezing point was equal to 0.68°, which, multiplied by 6, gives 4.08 per cent of sugar, with a rotation equal to 4 per cent. Thus, there can be absolutely no doubt that the factor which was first calculated on the theoretical basis and subsequently demonstrated to be correct, making use of diabetic urine, and normal urine to which sugar had been added, must be accepted; and the figures obtained by Fuchs bearing upon the subject of diabetic urine must be looked upon as erroneous. He gives 10 as the factor by which the difference between the two freezing points

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must be multiplied, which would, as may readily be seen, lead to serious error in each case presented in the tables accompanying this article; and further, this factor is not in agreement with the theory and calculations based upon the relative size of the molecules.

*Practical method of working.* — The method which we have adopted in the laboratory is as follows: The specific gravity of the urine is determined at 15° approximately, and any variation in temperature allowed for by adding 0.0001 for each 1° C. above 15°. The freezing point of the urine is determined, and at the same time the urine is polarized. The theoretical freezing point is calculated from the specific gravity by multiplying the portion of the specific gravity after the decimal point by 75, the observed freezing point is subtracted from this figure, and the difference in degrees Centigrade is multiplied by 6, which gives very approximately the percentage of sugar in the urine. The results agree very closely with those found by other methods, such as polarization, provided there is nothing present in the urine which interferes with the other methods. So far as I am able to say, this method is less influenced by external conditions than any other. It is also possible to make a determination in a very short space of time, as many as ten or twelve such determinations having been made in a single hour. It is very surprising that radical changes in diet do not affect the result very materially, although I have on occasion observed slight error due to the treatment of diabetic cases on a restricted diet, with large quantities of bicarbonate, which, possessing as it does much smaller ions than the average of those present in urine, should diminish the average ionic weight of the urine.

It should be borne in mind that this method is more especially applicable to urine containing a large percentage of sugar; for, the larger the proportion of sugar in a urine, the smaller is the proportion of ordinary urine solids. In fact, a point is often reached at which the normal constituents are so distributed in a large bulk of urine that, were the sugar removed, the specific gravity due to their influence would not be more than 1.002 or 1.003. In urines of very slight concentration, less than half a per cent, it is hardly safe to depend upon this method for absolute results, although very satisfactory for a series of comparative results from day to day. Above 2 per cent up to 7 and 8 per cent, the method was found to be accurate with a maximum experimental error of 0.3 to 0.4 per cent, the average variation not being as much as 0.1 per cent.

As stated above, we have, in carrying out this work, refrained from

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introducing corrections for sub-cooling, atmospheric conditions, etc., whereby the experimental error might be reduced in a physicochemical laboratory to an almost negligible quantity. The method may be employed, as stated above, with fairly accurate results, in any physiological chemical laboratory, or even in the clinical laboratory of hospitals. In studying and treating severe cases of diabetes, it is of the greatest value to obtain accurate information as early as possible, and in that respect we have obtained the most satisfactory results by combining this method and that of polarization, confirming our results from time to time by one of the other methods.

One point of especial importance might be mentioned at this stage. Betaoxybutyric acid, when present in urine, interferes very perceptibly with rotation of the plane of polarized light, causing the dextrose to show a lower figure than would be anticipated from the actual percentage of sugar present in the solution. When large quantities of this acid are present, the freezing point results deviate from the rotation figure. In fact, the agreement of the results obtained by these two methods for a period of several days justifies the assumption that the acid in question is absent. This fact alone is of considerable importance, as the fermentation method usually employed, and subsequent examination for betaoxybutyric acid, involves a considerable expenditure of time. We have, in presenting cases, purposely chosen those which showed large quantities of sugar with only traces of the oxyacids, in order not to complicate the problem to too great an extent. We have, however, had under observation cases in which considerable quantities of the oxyacids were present, sufficient to exert a more than negligible effect upon the rotation and freezing point. We have not, however, ventured to make use of this difference as a means of estimating the quantity of oxybutyric acid, owing to the fact that the sources of experimental error are so numerous in such a case as to make its use out of the question, except when large quantities of the acid are present, which is seldom the case.

**Estimation of albumin.** — The quantitative estimation of albumin in urine is extremely difficult to carry out accurately; and since it is possible, in the majority of cases, to form a fair idea of the actual amount of coagulable albumin present in urine, by our method, we propose to give it in outline, although it has not been as thoroughly investigated or proved as serviceable as has the method of estimating sugar.

Whilst the sugar molecule is 180, as compared with the 57 to 60 of

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urine solids, the molecular weight of albumin is so large as to be looked upon as infinitely great when compared with the 60 of urine solids referred to above. That is to say: the effect produced upon the freezing point by albumin should be so small as to be negligible, especially in view of the fact that it is seldom present in urine in larger quantities than 1 per cent. This does not, however, apply to the specific gravity, which is influenced by albumin, when actually in solution, just as it is by other solids. Bearing this in mind, we tried the effect of adding a couple of drops of acetic acid to the filtered urine, determining the specific gravity and freezing point, then boiling the urine in order to precipitate the albumin, which would be thrown out of solution by means of dilute acetic acid, and once more determining the freezing point and specific gravity in the clear centrifuged portion.

Boiling the urine in order to precipitate the albumin, and centrifuging in order to remove the precipitated solids, leads to an increase in its concentration sufficient to interfere materially with any calculation, based upon the loss of specific gravity effected by the removal of the albumin, unless we have some means at our disposal of reducing the second specific gravity to the same basis of ion concentration as the first. The slight change in specific gravity would not be sufficient to interfere materially with the degree of dissociation of the salts, and although the removal of albumin should lead to an increased activity on the part of certain ions, others have probably been completely removed in combination with the proteid. These factors, however, appear from the results obtained to counterbalance one another, and may, therefore, be neglected. Our procedure is as follows:

The decimal portion of specific gravity determined after the addition of a drop or two of acetic, but before boiling, is designated $S_1$. The freezing point determined in the same urine at the same time is represented by $\Delta_1$. After boiling and centrifuging, the decimal portion of the specific gravity and the freezing point, once more determined, are indicated as $S_2$ and $\Delta_2$. Then, in order to reduce $S_2$ to the same ionic concentration as $S_1$, the former is multiplied by $\Delta_1$ and divided by $\Delta_2$. This quantity is then subtracted from $S_1$, which gives that portion of the specific gravity figure which may be attributed to the influence of the bodies precipitated by boiling (albumin). It has been found that each 0.01 of specific gravity is equivalent to 3.8 or 4 per cent of albumin. The difference obtained above is therefore multiplied by 400 in order to obtain the percentage of albumin. Table XII gives a series of results obtained in this way, in which a very

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fair agreement may be observed between the method in question and other accepted methods which require a much larger expenditure of time.

As this method is somewhat complicated, it will be simpler to illustrate it by means of an example. In Case 1, the specific gravity, determined at 15° C., by means of a very accurate Westphal balance,

TABLE XII.

ESTIMATION OF ALBUMIN (IN URINES, ETC.).

| Case. | $S_1$. | $S_2$. | $\Delta_1$. | $\Delta_2$. | $\frac{S_2\Delta_1}{\Delta_2}$. | $S_1-\left(\frac{S_2\Delta_1}{\Delta_2}\right)$. | Albumin Diff. × 400. | Albumin by other methods. |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | per cent. | per cent. |
| 1 | 0.0261 | 0.0262 | 1.935 | 2.010 | 1.0252 | 0.0009 | 0.36 | 0.35 |
| 2 | 0.0161 | 0.0173 | 1.265 | 1.420 | 0.0154 | 0.0007 | 0.28 | 0.30 |
| 3 | 0.0153 | 0.0164 | 1.165 | 1.285 | 0.01487 | 0.00043 | 0.17 | 0.20 |
| 4 | 0.0075 | 0.0052 | 0.35 | 0.405 | 0.0045 | 0.0030 | 1.20 | 1.00 |
| 5 | 0.0211 | 0.0225 | 1.47 | 1.63 | 0.0203 | 0.0008 | 0.32 | 0.35 |
| 6 | 0.0135 | 0.0139 | 1.04 | 1.10 | 0.01314 | 0.00036 | 0.14 | 0.13 |
| 7 | 0.0223 | 0.0223 | 1.62 | 1.74 | 0.0207 | 0.0016 | 0.64 | |
| 8 | 0.0205 | 0.0212 | 1.51 | 1.56 | 0.0205 | nil | nil | 0.20 |
| 9 | 0.0259 | 0.0283 | 1.915 | 2.09 | 0.0259 | nil | nil | nil |
| EXUDATE CANCER CASE K. | | | | | | | | |
| | 0.0117 | 0.0082 | 0.525 | 0.625 | 0.0070 | 0.0047 | 1.88 | 1.70 |
| EGG ALBUMIN SOLUTION. | | | | | | | | |
| | 0.0197 | 0.0030 | 0.245 | 0.16 | 0.0046 | 0.0151 | 6.04 | 6.00 |

was 1.0261 before, and 1.0262 after boiling. The freezing points were respectively 1.935 and 2.010 before and after boiling. The factor $\frac{S_2\,\Delta_1}{\Delta_2}$ is consequently equal to 0.0252. 0.0252 subtracted from 0.0261, the decimal portion of the original specific gravity, gives 0.0009, which, multiplied by $400 = 0.36$ per cent.

It must be mentioned at this stage, that whilst a large proportion of determinations give accurate results, the possibilities of error are considerable, and in certain cases we have been unable to explain deviations from the correct figure, such as that exhibited in Case 8, in the table. If accurately and carefully handled, such a method should be of considerable use, especially from a theoretical standpoint, and in

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those cases in which we have to deal with a large percentage of albumin, the experimental error should be practically negligible, if the precaution is taken of making both specific gravity determinations at the same time, and at exactly the same temperature, and using the same thermometer for the freezing point determinations in two successive experiments.

A normal urine free from albumin has been included in the table as Case 9, in order to show that there is nothing in normal urine to interfere with satisfactory results by this method. We have also included two cases of albumin solutions of high concentration, one an exudate in a cancer case containing 1.75 per cent of albumin, and the other a solution of egg white in water, containing six parts of albumin. These solutions were handled in exactly the same way as the urines, the second specific gravity being reduced to the same ionic concentration as the first. There are, of course, very disturbing factors in this case, owing to the removal of certain salts by the precipitated proteids and to the neglect of the influence exerted upon the freezing point by the proteids themselves. Consequently, the close agreement in the results obtained from the egg-white solution can only be looked upon as accidental.

This method is merely offered in the form of a suggestion, as having possible application for purposes of confirmation and also in experimental work. We should not recommend it for daily use in the laboratory, as has been done in the case of the method employed for the estimation of sugar.

**Analysis of urines containing both sugar and albumin.** — It will be seen from the above that albumin and sugar may both be estimated in the same urine by means of the freezing point method. A few drops of acetic acid are added, $S_1$ and $\Delta_1$ determined, the urine is boiled, and $S_2$ and $\Delta_2$ obtained. The albumin is estimated, as described in the last section, by combination of these factors, and the sugar may be determined, making use of $S_2$ and $\Delta_2$, calculating the theoretical freezing point from $S_2$, deducting $\Delta_2$ from the result, and multiplying the figure obtained by 6. The few drops of acetic acid added exert no appreciable effect upon the estimation of sugar by this method. In fact, it may be said that albumin itself, when present in small quantities, exerts so slight an effect as not materially to interfere with the direct estimation of sugar in urine.

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## Summary.

1. In normal urine the depression of the freezing point is directly proportional to the specific gravity, and may be determined from the latter by multiplying the figures after the decimal point by 75. (As an example, if the specific gravity is 1.022, $0.022 \times 75 = 1.65$, which is found by experiment to be the depression of the freezing point of such a solution in degrees C.) (See Tables I and II.) This portion of the work is in agreement with the findings of Fuchs.

2. In pathological urines other than those containing sugar and albumin, the same tendency to maintain a constant proportion between the freezing point and specific gravity may be observed, although within rather wider limits than in the case of normal urines. (See Tables III and IV.)

3. The average weight of the molecule or ion in urine may be determined from these figures as approximately 56 to 60, making use of Beckmann's formula,

$$M = E \frac{m}{\Delta},$$

where $M$ = molecular weight of substance dissolved; $E$ = a constant having the value of 18.5 when water is employed as the solvent; $m$ = the quantity of substance dissolved, assumed in this case to be 2.33 grams of substance for each 0.01 of specific gravity; and $\Delta$ = the observed depression of the freezing point ($0.75°$ for each 0.01 of specific gravity).

4. In cancer, typhoid fever, and other diseases in which the excretion of chlorides is extremely small, the effect exerted upon the freezing point depression is not so great as might be expected from the removal of such a large proportion of the smaller ions. This tendency to maintain a constant state of equilibrium in the average size of the ions and molecules present in the urine may account for the high retention of the products of sub-oxidation and abnormal metabolism, known to exist in these cases. This view of the case may be advanced as a further argument in favor of the therapeutic administration of chlorides to patients on a milk diet, in order that molecular equilibrium of the urine may be maintained, and a more complete elimination of the products of metabolism effected.

5. In diabetic urines, the quantity of sugar may be very readily estimated by determining the lowering of the freezing point of the

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urine in question, and calculating the theoretical lowering of the freezing point from the specific gravity. The difference between these two quantities in degrees C., when multiplied by 6, gives a very close approximation of the actual percentage of sugar present in the solution. This factor, 6, calculated on a theoretical basis (see footnote, page 331), and subsequently confirmed in practice, is dependent on the fact that the molecular weight of sugar is 180, whilst the average of urine solids is below 60. (See Tables VI, VII, VIII, IX, X, and XI.) These results are not all in accord with the observations made by Fuchs, who arrived at his factor, 10, by purely empirical means.

6. The quantity of albumin may be estimated by determining the specific gravity and freezing point in a urine to which a couple of drops of dilute acetic acid has been added, then boiling in order to precipitate the albumin, filtering and once more determining the specific gravity and freezing point. The second specific gravity is reduced to the same ionic concentration as the first, by multiplying its decimal portion by the freezing point before boiling, and dividing by the freezing point obtained after boiling. This calculated specific gravity is then subtracted from the original specific gravity determined before boiling, and the difference multiplied by the factor 400, which gives the percentage of albumin. (See Table XII.)

7. In a urine containing both albumin and sugar, the former is first estimated by addition of acetic acid, as above, and the freezing point and specific gravity obtained after boiling are employed in the estimation of sugar.

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# NEW EXPERIMENTS ON THE PHYSIOLOGICAL ACTION OF THE PROTEOSES.

By FRANK P. UNDERHILL.
(Research Scholar of the Rockefeller Institute.)

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

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## INTRODUCTORY.

THE attempt to follow the fate of ingested proteids in their passage from the alimentary canal was the occasion for the original studies on the behavior of proteolytic digestion products introduced directly into the circulation. Since the early experiments in Ludwig's laboratory numerous investigators have devoted their attention to this subject.[1] The physiological reactions called forth by intravenous injections of such materials — the "peptone" of earlier writers — are diverse, and include the rendering of the blood incoagulable together with changes in its reaction and composition; an acceleration of lymph-flow; a fall in arterial pressure; anuria; deep narcosis; and other toxic symptoms, as well as a certain degree of immunity toward subsequent injections.

[1] The earlier literature is reviewed in the paper by CHITTENDEN, MENDEL, and HENDERSON: This journal, 1898–99, ii, p. 142; see also Studies in Physiological Chemistry, Yale University, 1901, p. 279.



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The reactions just described are not common to all species of animals; or at any rate they are not obtained with equal readiness and intensity. The organism of the dog is particularly susceptible to the effects of intravenous injections of the products of proteolysis. In the cat the characteristic symptoms are evoked somewhat less readily, larger doses being necessary to produce comparable results. The rabbit, on the other hand, is extremely resistant, and, except in rare cases, fails to respond in so far as the phenomena involving the blood are concerned. Quite recently Persano[1] has found that although commercial "propeptone" preparations are fatal to guinea pigs, they fail to render the blood non-coagulable except when injected rapidly into fasting animals. Persano also secured positive results with the toad (Bufo vulgaris and B. viridis) and the tortoise (Emys europæa). In Elasmobranchs Bottazzi[2] noted an effect on coagulation with 0.5–0.8 gm. "peptone" per kilo; and in those marine invertebrates, the blood of which does not ordinarily clot, peptone injections were found to bring about abnormal changes of another order.

It is well, perhaps, to emphasize these variations in the behavior of different species, since they have at times been overlooked in comparing the experimental results of different investigators. Thus Fiquet[3] noted that various digestive products which were purified by methods of reprecipitation suggested by Gautier were apparently harmless when injected intravenously in relatively large quantities into rabbits. Nencki[4] has taken occasion to call attention to the importance of Fiquet's work in reference to future studies on the non-coagulation of "peptone-plasma;" and Pick and Spiro[5] have attached great importance to the negative results noted. All of these investigators seem to have overlooked the natural immunity of rabbits to injections of proteoses. Moreover it is said to be possible to produce a noticeable retardation of blood-coagulation even in rabbits when sufficiently large doses are rapidly injected.[6] All investigators are agreed with reference to one experimental factor, namely, the

[1] PERSANO: Archives italiennes de biologie, 1902, xxxvii, p. 409.

[2] BOTTAZZI: *Ibid.*, 1902, p. 63.

[3] FIQUET: Comptes rendus de la société de biologie, 1897, xlix, p. 459.

[4] NENCKI: Jahresbericht für Thierchemie, 1897, xxvii, p. 196 (foot-note).

[5] PICK and SPIRO: Zeitschrift für physiologische Chemie, 1900–01, xxxi, p. 237.

[6] *Cf.* NOLF: Bulletin de l'Académie royale Belgique (Classe des sciences), 1902, No. 11, p. 866, foot-note 1.

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necessity of making the injections rapidly.[1] Only under such conditions are the typical phenomena provoked in even the most susceptible animals. "It seems reasonable to assume that the intensity of vaso-motor effects is not so much a function directly of the absolute quantity injected as of the quantity in the circulation per unit of time, or, in other words, of the concentration of the substance in the blood."[2] Finally the dosage is not without considerable importance in determining the intensity and duration of the effects obtainable. In general the proteoses provoke a marked response in doses of 0.3–0.5 gm. per kilo of body-weight in dogs. According to Thompson[3] doses of Witte's peptone as low as 0.02 gm. per kilo may retard blood-coagulation. Smaller doses produce contrary effects, coagulation being accelerated; while less than 0.0075 gm. per kilo is entirely without influence. Rapidly injected doses of 0.010–0.015 gm. per kilo never fail, according to Thompson, to produce a fall in blood-pressure.

It was to the phenomena provoked that the earlier investigators turned their attention ; and little consideration was given to the more careful isolation or chemical differentiation of the materials injected. The latter, briefly designated "peptone," were essentially a mixture of the neutralized products obtained by the gastric (or sometimes pancreatic) digestion of crude fibrin or egg-white. With the progress in the study of digestive proteolysis came the endeavor to ascertain the physiological influence of better characterized products. The literature of this subject has been reviewed in detail in an earlier paper from this laboratory ;[4] it will suffice here to refer to the contributions of Pollitzer,[5] Grosjean,[6] Arthus and Huber,[7] Thompson,[8] Ledoux,[9] Spiro and Ellinger,[10] Chittenden and Mendel associated with Henderson[11] and McDermott.[12] To these may be added the very

[1] *Cf.* NOLF: *Loc. cit.*, p. 871 and 883.
[2] CHITTENDEN, MENDEL, and HENDERSON: This journal, 1898–99, ii, p. 150.
[3] THOMPSON: Journal of physiology, 1896, xx, p. 455.
[4] *Cf.* CHITTENDEN, MENDEL, and HENDERSON: *Loc. cit.*, p. 142.
[5] POLLITZER: Journal of physiology, 1886, vii, p. 283.
[6] GROSJEAN: Archives de biologie, 1892, xii, p. 1.
[7] ARTHUS and HUBER: Archives de physiologie, 1896, viii, p. 857.
[8] THOMPSON: Journal of physiology, 1896, xx, p. 455; also 1899, xxiv, p. 374.
[9] LEDOUX: Archives de biologie, 1896, xiv, p. 63.
[10] SPIRO and ELLINGER: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 121.
[11] CHITTENDEN, MENDEL, and HENDERSON: *Loc. cit*, p. 142.
[12] CHITTENDEN, MENDEL, and MCDERMOTT: This journal, 1898, ii, p. 255.

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recent experiments of Nolf[1] which have been published since the completion of our work. Without repeating the particulars of the various investigations they may be said to indicate that pronounced differences exist between the physiological action of the group of proteid substances known collectively as proteoses (propeptones) and that of the peptones — the latter being substances not precipitable by ammonium sulphate. Compared with the proteoses the peptones are relatively inert in the directions specially indicated in this paper. Furthermore the proteoses differ in respect to their efficiency in producing toxic symptoms. Thus according to several of the investigators heteroproteose is more active physiologically than some of the other fractions obtainable by the modern methods of proteid differentiation (precipitation by salts, dialysis); while other products like gelatoses and caseoses seem to act only in large doses. Earlier studies in this laboratory[2] have shown that typical results can be obtained not only with products of peptic proteolysis, but also with proteoses prepared with the vegetable enzyme papaïn or even by the action of acids alone. Finally Nolf[3] has undertaken a large number of experiments with proteoses (propeptone) prepared from fibrin by peptic digestion, by pancreatic digestion, and by autolysis. In the latter case the proteolysis is attributed to the presence of an enzyme derived from the leucocytes entangled in the fibrin. His experimental study has demonstrated that under the conditions noted (namely, rapid injections of larger doses in the dog) all these proteoses retard the coagulation of the blood and lower arterial pressure. The peptic products, however, are relatively more toxic in this respect. Such variations are doubtless attributable to differences in the relative proportions of different specific proteoses. That the latter should differ in their physiological action, even though they are derived from the same mother substance, need not surprise us in view of the apparent chemical dissimilarities which recent investigation has revealed in regard to composition of the proteoses.[4] Indeed it is only to be expected that with the refinement of chemical methods for the separation of proteoses, the well-defined individuals of this group of proteid compounds will be found to react in specific ways in the organism.

[1] Nolf: *Loc. cit.*
[2] Chittenden and Mendel, with Henderson and McDermott: *Loc. cit.*
[3] Nolf: *Loc. cit.*
[4] *Cf.* Pick: Beiträge zur chemischen Physiologie, 1902, ii, p. 509.

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It should be pointed out in this connection that the characteristic physiological reactions noted after proteose injections can also be obtained with a variety of other substances of known or partly known composition. This statement applies, for example, to the nucleic acids, as has been demonstrated in a recent investigation by Mendel, Underhill, and White in this laboratory.[1] The doses required are far smaller than in the case of the proteoses, 0.05 gm. per kilo of body-weight sufficing to bring about a noticeable fall in arterial pressure, lymphagogic effects, and retardation of coagulation in the case of the nucleic acid of the wheat embryo (tritico-nucleic acid). Similar results were previously noted by Bang,[2] who injected pancreas nucleoproteid. In addition to this may be recalled the older observations on lowered arterial pressure and retardation of blood-coagulation induced by injections of various enzymes,[3] bacterial and tissue products,[4] snake venoms,[5] protamins,[6] etc. Many of these products also act in extremely small doses. But this fact, by itself, will not justify the assumption that in view of the larger doses of proteose necessary to produce comparable symptoms, the physiological action of the proteoses is due to some highly toxic contaminating substance like those referred to. Otherwise one might, for example, as well attribute the tetanic action of large doses of brucin preparations to the presence of small quantities of adherent strychnine which acts in far smaller doses than its companion alkaloid.

Although the toxic properties of the proteoses have been generally accepted as a specific characteristic of their behavior after direct introduction into the circulation, this view has lately been subjected to severe criticism in Hofmeister's laboratory by Pick and Spiro.[7] Those investigators demonstrated, in the first place, that enzymes

[1] MENDEL, UNDERHILL, and WHITE: This journal, 1903, viii, p. 377. The literature is cited in this paper.

[2] BANG: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 201.

[3] *Cf.* ALBERTONI: Centralblatt für die medicinische Wissenchaften, 1878, xvi, p. 641; SALVIOLI: *Ibid.*, 1879, xvii, p. 596; DASTRE and FLORESCO: Comptes rendus de la société de biologie, 1897, xlix, p. 847.

[4] CHITTENDEN: Digestive proteolysis, 1895, p 90.

[5] WOLFENDEN: Journal of physiology, 1886, vii, pp. 327, 357, 365; KANTHACK: *Ibid.*, 1892, xiii, p. 272; MARTIN: *Ibid.*, 1893, xv, p. 380; also Proceedings of the Royal Society of New South Wales, 1892.

[6] THOMPSON: Zeitschrift für physiologische Chemie, 1900, xxix, p. 1.

[7] PICK and SPIRO: *Loc. cit.*

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can be dispensed with in the production of physiologically active mixtures from proteid substances. This was, however, quite to be expected after Chittenden, Mendel, and Henderson had shown that products obtained by the hydrolysis of egg-albumin with sulphuric acid induce a pronounced physiological reaction, as well as those formed from the same proteid by pepsin digestion.[1] With the products of pancreatic digestion of fibrin Pick and Spiro failed to obtain any alteration in the coagulability of the blood, and they call attention to the similar failure of Fano[2] in earlier experiments. With reference to the actual proteose content of the "trypton" used by Fano nothing is known. Pick and Spiro state that the unpurified digestion mixture used by them contained "an abundance of albumoses precipitable by half-saturation with ammonium sulphate." The actual dosage of proteose in the single experiment recorded (Versuch III) was not ascertained nor is any statement made regarding the rate of injection. Chittenden, Mendel, and Henderson obtained pronounced reactions with isolated proteoses prepared by the action of pancreatic juice on "antialbumid;"[3] and in accord with this are the recent experiments of Nolf[4] with isolated "propeptone" obtained (by saturation with ammonium sulphate) from the products of the digestion of fibrin with pancreatic extracts. The products of autolysis of fibrin also failed to give positive results in the hands of Pick and Spiro. But here again no attempt was made to study the isolated proteoses, the authors contenting themselves with the statement in one experiment (VI) that the material injected "still contained small amounts of the albumoses precipitable by half-saturation with ammonium sulphate." In direct contradiction to these observations are the positive results obtained by Nolf with "propeptone" isolated from comparable material.[5] Nolf attempts to explain the negative results of the German investigators on the basis of his own experiments by assuming that they failed to make the intravenous injections with sufficient rapidity. From our experience we think it not unlikely that the *actual content of proteose* in Pick and Spiro's injection mixtures was smaller than they intended it to be. Furthermore, we have as yet no means of knowing to what

[1] CHITTENDEN, MENDEL, and HENDERSON: *Loc. cit.*
[2] FANO: Archiv für Physiologie, 1881, p. 277.
[3] CHITTENDEN, MENDEL, and HENDERSON: *Loc. cit.*
[4] NOLF: *Loc. cit.*
[5] NOLF: *Loc. cit.*

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extent the non-proteose constituents of mixtures such as they used may influence the reactions attributed to the proteoses. In fact, it will be shown in the experimental part of this paper that the property of producing the typical physiological effects is lost when purified proteoses are heated with dilute acid until the biuret reaction can no longer be obtained.

In their discussion of the question as to whether the clot-retarding agent in proteolysis mixtures is a proteose, Pick and Spiro conclude: "Albumose mixtures obtained by the action of trypsin, autolysis, or alkalies on crude fibrin have no action on blood-coagulation, although they can be demonstrated to contain albumoses which cannot, at the present time, be distinguished from those obtainable by the action of pepsin-hydrochloric acid or acid alone."[1] But as indicated in the preceding review, their evidence is as yet inadequate to maintain this assumption. In addition to the contradictory experience of Nolf and other criticism noted above, it must again be emphasized that Pick and Spiro made no attempt to inject the proteoses as such, prepared by the various methods referred to. This seems to us a matter of primary importance, however; and the criticism applies equally to the subsequent chapter in Pick and Spiro's paper in which they have discussed their failure to obtain anti-clotting substances by the hydrolysis of pure proteids (casein and edestin) with acids alone. Here again we must refer to the earlier experiences in this laboratory with the proteoses prepared from egg-albumin by the action of acid alone. The significance of these positive experiments does not seem to have impressed Pick and Spiro, although they quote the negative results obtained by Chittenden, Mendel, and Henderson in a trial of anti-albumid — not ordinary proteose — made from edestin. Pick and Spiro's method consisted in digesting purified casein, or edestin, a crystalline proteid from hemp-seed, with 0.8 per cent hydrochloric acid for several days in a thermostat. The solutions were neutralized, the acid proteid removed, and the filtrates dried on a water bath. Solutions of these resulting mixtures were then injected into dogs and the effects on the coagulability of the blood noted. The details reported show in one case that the injection was made in four minutes; in the other no data are given. The presence of proteoses was tested for by fractional saturation trials with ammonium sulphate. In the case of the edestin products the largest fraction was that obtained by one-half saturation. The isolated proteoses were not injected.

[1] Pick and Spiro: *Loc. cit.*, p. 250.

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In repeating these experiments under similar conditions we have been unable to obtain large quantities of proteoses in so short a time. And in this connection it may be well to point out the difficulty of separating out the acid proteid completely from such mixtures. How difficult it is to remove edestin itself from solutions by coagulation alone was pointed out long ago by Chittenden and Mendel;[1] and the precautions necessary for the complete separation of acid proteids have lately been indicated by Hawk and Gies.[2] Experiments which we have made according to the directions of Pick and Spiro have given us similar negative results. But, as will be seen later, the isolated proteoses prepared by the acid hydrolysis of pure proteid, (edestin, etc.) were found to exert the characteristic retarding action on blood-coagulation; and in those trials in which the hydrolysis was extended over a longer time than that suggested by Pick and Spiro and in which the content of proteoses was *actually determined*, positive results were likewise noted.

Pick and Spiro have attempted to demonstrate the non-toxicity of the proteoses in still other ways which involve, according to them, a purification of the proteid. They have found: (1) that the anti-clotting substance is destroyed by alcohol in weakly alkaline, but not in acid solutions; (2) that the anti-clotting substance is presumably not preformed in crude fibrin, but is liberated or manufactured by the action of acids. Fibrin would, then, contain a fore-runner, the mother-substance of the toxic substance; and the so-called physiological albumose-reactions are accordingly to be attributed to a contamination of the proteid — of the fibrin used, in the case of commercial "peptones." In further support of this view the authors find that anti-clotting extracts (free from proteoses) can be prepared from various tissues of the body, viz., the gastric and intestinal mucosa and the pancreas; while negative results are obtained with albumose-containing extracts of testicles, thymus, suprarenals, submaxillary and lymphatic glands, and œsophageal membrane. In formulating their explanation it has apparently not occurred to Pick and Spiro that there may be (and probably are) *different* anti-clotting substances in various tissues of the body; nor have they made mention of the possible presence of compounds (like some nucleoproteids) which *facilitate* coagulation and may act antagonistically to the proteoses. The presence of non-proteid anti-clotting compounds in

[1] CHITTENDEN and MENDEL: Journal of physiology, 1894–95, xvii, p. 48.

[2] HAWK and GIES: This journal, 1902, vii, p. 460.

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the tissues can surely not be taken *per se* as evidence of the inactivity of proteoses prepared from tissue proteids. In the case of the pancreas, for example, the anti-clotting power of its constituent nucleic acid compounds has already been mentioned. For the anti-clotting substance (or substances) derived from the tissues, the name "peptozyme" has been proposed, its antecedent being designated as "peptozymogen." Peptozyme is, then, a substance very resistant to the action of dilute mineral acids and to heating in neutral or faintly alkaline solutions; and since its antecedents are present in many of the animal tissues, proteoses derived from the latter by digestion must be contaminated with it. Thus preparations made with pepsin may contain active peptozyme derived from the gastric mucosa; fibrin products like "Witte peptone" are doubly contaminated. In attempting to account for the positive results which Chittenden, Mendel, and McDermott[1] obtained with proteoses prepared by digestion of coagulated egg-white with papaïn in *alkaline* media, Pick and Spiro have made the purely hypothetical assumption that the peptozymes (derived from the egg?) are resistant to papaïn. Hypothetical as much of this is, it is by no means impossible or even improbable. But misguided by their inadequate experiments with pure proteids, etc., discussed above, the authors have gone a step further and defend the theme: "Es gibt 'Peptone' ohne 'Peptonwirkung' und 'Peptonwirkung' ohne 'Peptone'" (p. 272).

The latter part of this proposition no one will deny. We propose to examine somewhat more closely the statement that the proteoses *per se* are physiologically inert in so far as their action on the coagulation of the blood and on arterial pressure is concerned. Pick and Spiro have summarized their position as follows: "It is possible to obtain by proteolysis (*e.g.* by trypsin, autolysis, alkali; with casein and edestin by acid also) typical albumoses and peptones which fail to show any influence upon blood-pressure when they are injected into the blood. But even the typical active products prepared by acid or pepsin-acid methods, lose this activity by a relatively harmless purification (treatment with alcohol) without undergoing a change in chemical character" (p. 271–272).

In the present research proteoses have been prepared from a number of purified proteids of different origin by various methods of hydrolysis, the presence of hypothetical anti-clotting compounds being excluded as far as our present knowledge and methods would

[1] CHITTENDEN, MENDEL, and McDERMOTT: *Loc. cit.*

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permit. In addition to this, many of the experiments of Pick and Spiro have been repeated in the hope of arriving at an explanation of the discrepancies which have arisen.

## Technique of the Experiments.

The experiments were carried out upon full-grown dogs which were always made to fast for a day previous to the experiment. Narcosis was induced by a subcutaneous injection of a mixture of morphine and atropine sulphates,[1] and when necessary, as during the operative procedures, complete anæsthesia was maintained by the administration of A. C. E. mixture. Arterial pressure was recorded from the left carotid artery with a mercurial manometer. The injections were made at a temperature of 38° C. into the right femoral vein from a burette. The blood samples were drawn from the left femoral artery, a clean, dry cannula being used for each sample. A small portion of blood was always allowed to flow through the cannula before the samples (2–3 c.c.) were collected in dry, graduated cylinders of uniform calibre. The rate of coagulation was taken to be the time which elapsed before the cylinder could be inverted without the loss of a drop. The substances injected were dissolved either in 0.7 per cent sodium chloride solution or in distilled water to which enough sodium carbonate was added to give a weak alkaline reaction, the volume used being equivalent, where possible, to 4 c.c. per kilo of animal. The injections were made rapidly — usually within a minute.

Unless otherwise stated, the proteoses used were obtained by saturation with ammonium sulphate after neutralization of the digestive mixtures and removal of undigested residues and the neutralization precipitate. The precipitates of proteoses were re-dissolved, dialyzed until *entirely* free from ammonium sulphate, concentrated, precipitated with alcohol, washed with hot alcohol, and finally dried with ether.

## Intravenous Injections of Typical Native Proteids.

Heidenhain[2] in his experiments on lymph-formation, has, in one case in which a native proteid was injected, noted that it had a

[1] The usual dose employed was ten milligrams of morphine sulphate and one milligram of atropine sulphate per kilo of animal.

[2] Heidenhain: Archiv für die gesammte Physiologie, 1891, xlix, p. 209.

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lymphagogic effect and yielded a non-coagulable lymph. Spiro[1] showed, however, that the injection of crystallized serum albumin is without influence. In the present investigation intravenous injections have been made with crystallized egg-albumin, ovomucoid, and the vegetable proteid excelsin obtained from the brazil-nut, *Bertholletia.* A typical protocol follows.

*Experiment 18.* — A bitch received an injection (82 c.c.), containing 4 gms. (0.5 gm. per kilo) of crystallized *egg-albumin.*[2] The reaction of the solution was slightly acid to litmus. The injection lasted 50 seconds. No influence was noted upon blood-coagulation; nor was there any fall in blood-pressure.

That the animal was not "immune"[3] follows from the fact that a subsequent injection (32 c.c.) of 4 gms. (0.5 gm. per kilo) of so-called *hemialbumose*[4] (made by the action of acid upon egg-albumin), lasting 75 seconds, completely suspended coagulation and lowered the pressure from 155 mm. to 20 mm. of mercury. At the end of an hour it had risen to 35 mm. Deep narcosis followed, together with marked intestinal peristalsis.

Similar experiments carried out with ovomucoid and with excelsin (in doses of 0.5 gm. per kilo body-weight) led to identical results.

It follows from these experiments that the typical native proteids may be injected rapidly without influencing the coagulation and arterial pressure of the blood, even when they are introduced in considerable quantity.

## Intravenous Injections of Proteoses prepared by the Digestion of Proteids of Animal Origin with Vegetable Enzymes.

The only experiments that have been carried o t to determine the influence of injections of proteoses prepared by the digestion of animal

[1] Spiro: Archiv für experimentelle Pathologie und Pharmakologie, 1898, xli, p. 148.

[2] Prepared according to Hopkins and Pinkus: Journal of physiology, 1898–99, xxiii, p. 130.

[3] By "immune" is meant the natural peculiarity of an animal, in that substances which generally have certain effects upon a species are without influence upon certain individuals. Temporary immunity may be established towards proteoses; for example, an injection of proteoses usually renders a subsequent injection ineffective, provided the time which has elapsed between the two injections is not too long.

[4] Chittenden, Mendel, and Henderson: This journal, 1898–99, ii, p. 173.

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proteids with vegetable enzymes are those of Chittenden, Mendel, and McDermott.[1] These investigators observed that proteoses and peptones prepared from egg-albumin by the action of papaïn behaved like products formed by pepsin. Since Pick and Spiro assume that the toxic effects noted after injection of proteoses are due to a contaminating substance derived from the animal tissues, and that these effects are not obtained when casein-acid products are employed, it was of interest to determine whether caseoses prepared by the agency of plant enzymes are non-toxic. Such injections have been made with bromelin and "Papoid" caseoses. These products were prepared by the action of bromelin — the proteolytic enzyme of the pineapple — and "Papoid" — a commercial papaïn preparation, on casein in *alkaline* media.

*Experiment 5.* — A bitch of 5 kilos received an injection (20 c.c.) of 5 gms. (1.0 gm. per kilo) of *caseoses* prepared with bromelin.[2] The reaction of the fluid was faintly acid. The injection lasted 40 seconds. The animal remained quiet. The following figures from protocols are given merely to indicate the method of observation employed in the numerous experiments to be reported. The average[3] clotting time of the blood before the injection was 8 minutes.

| Sample collected after | Time of clotting. | Blood-pressure in mm. mercury. | |
|---|---|---|---|
| 1 min. | ....[1] | Before injection .... | 140 |
| 3 min. | .... | At end of injection ... | 55 |
| 8 min. | .... | 2 min. after injection ... | 64 |
| 23 min. | .... | 3 min. after injection ... | 98 |
| 4 hrs. 22 min. | 2 hrs. 27 min. | 5 min. after injection ... | 114 |
| | | 7 min. after injection ... | 130 |
| | | 4 hrs. 22 min. after injection | 130 |

[1] Had not clotted in twenty hours.

It may be concluded, therefore, that injections of caseoses formed by the agency of vegetable enzymes provoke the effects typical for other proteoses.

[1] CHITTENDEN, MENDEL, and McDERMOTT: *Loc. cit.*
[2] Prepared according to CHITTENDEN: Journal of physiology, 1894, xv, p. 459.
[3] The average of three or four samples.

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## ARE PROTEOSES PREPARED FROM PROTEIDS AND ENZYMES, BOTH OF VEGETABLE ORIGIN, NON-TOXIC?

If the hypothetical "peptozyme" of Pick and Spiro is truly a contamination derived from the animal organism and is not present in the vegetable kingdom, as their results would seem to indicate, proteoses prepared from the vegetable proteids with an enzyme also of plant origin might be expected to give negative results when injected into the blood stream. In the absence of experiments on this subject recorded in the literature, trials were made with products prepared from crystallized edestin (of hemp-seed) by the action of bromelin and papaïn.

*Experiment 10.* — A dog received an injection (22 c.c.) of 2.75 gms. (0.5 gm. per kilo) of *edestin proteoses* prepared in the usual manner from a digestion of crystallized edestin by Merck's *papaïn* in an alkaline medium. The reaction of the fluid was faintly acid, and the injection lasted 30 seconds. There was evidence of great excitation (cries, struggles, etc.), together with labored breathing. Defæcation also occurred. Later deep narcosis appeared.

The average clotting time of the blood before the injection was 10 minutes.

The samples of blood collected at short intervals for 2 hours following the injection did not clot within 24 hours. A sample collected 2 hours 4 minutes after the injection clotted in 22 minutes. The blood-pressure showed the typical fall followed by a gradual rise.

A subsequent injection of "Witte's peptone" (0.3 gm. per kilo) caused a fall in pressure comparable with that of the first injection, except that it returned to the normal more speedily.

Proteoses prepared from edestin with bromelin and injected in doses of 1.3 gms. per kilo provoked effects similar to those observed for proteoses prepared from edestin with papaïn.

Since the proteoses employed in these experiments were entirely free from contaminating substances of animal origin, and further, since according to Pick and Spiro the toxic body, "peptozyme," is not developed in an alkaline medium (as in pancreatic digestion), we attribute the results to the proteoses themselves.

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## Intravenous Injections of Proteoses prepared from Vegetable Proteids by Hydrolysis with Acids or with Superheated Water.

Chittenden, Mendel, and Henderson[1] have noted that albumoses prepared by hydrolysis with acids exert a very toxic influence when injected. Pick and Spiro later observed similar effects after injection of products obtained from fibrin with acids. With their product prepared from edestin no such results were noted, the coagulability of the blood and the arterial pressure remaining unchanged. No experiments were carried out with the *isolated* proteoses, however, the products employed being mixtures of substances remaining after removal of the neutralization precipitate.

As has been noted above, *excelsin*, a simple proteid obtained from the brazil-nut, is without influence upon the coagulability of the blood or arterial pressure. If this proteid be hydrolized with dilute hydrochloric acid and the resulting proteoses separated, the physiological effects produced by them resemble those called forth by other proteoses. Furthermore proteoses prepared by the action of sulphuric acid on edestin and zeïn[2] provoke similar effects.

*Experiment 46.* — The animal received an injection (34 c.c.) of 4.25 gms. (0.5 gm. per kilo) of *proteoses*, prepared by the action of 0.8 per cent hydrochloric acid on *excelsin* for 20 hours in a sterilizer, and separated in the manner usually employed in this investigation. The reaction of the solution was faintly alkaline, and the injection lasted 45 seconds. There was evidence of great excitation (cries, struggles, etc.), followed by deep narcosis. Increased intestinal peristalsis was also noted.

The average clotting time of the blood before the injection was 14 minutes. The samples of blood collected at frequent intervals for 2 hours after the injection showed no tendency to clot 24 hours later. The usual fall in pressure was observed.

Injections of proteoses formed from zeïn[3] and edestin (by the action of 2 per cent sulphuric acid in a sterilizer) in doses of 0.2-0.9

[1] Chittenden, Mendel, and Henderson: *Loc. cit.*

[2] Szumowski: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 198. This investigator has shown that injections of zeïn itself provoke a retardation of blood-coagulability and a fall in arterial blood-pressure. The effects are, however, not so marked as with the injection of proteoses from zeïn.

[3] The zeïn was prepared as follows: Corn meal from which the starch had been removed was extracted with 95 per cent alcohol. The extract was filtered

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gm. per kilo either retarded or entirely inhibited the blood-coagulation. The typical phenomena connected with the blood-pressure were always present.

The following experiment with proteoses, prepared from edestin by the action of superheated water in the autoclave, and separated in the usual manner, was also carried out.

*Experiment 14.* — A bitch of 7.5 kilos received an injection (50 c.c.) of 2.75 gms. (0.36 gm. per kilo), of *edestin-proteoses* in a faintly alkaline solution. The injection lasted 67 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 9½ minutes.

Blood samples collected 1 min., 6 min., 32 min., and 1 hour 12 min. after the injection, clotted in 3 hours, 2 hours 37 min., 27 min., and 12 min. respectively. The usual fall in the pressure of the blood was also observed.

These experiments suggest the conclusion that proteoses resulting from the hydrolysis of vegetable proteids may exert a toxic influence in the manner described.

## The Physiological Action of Native Proteoses.

In view of the observations recorded above, showing that typical native proteids exert no influence upon either blood-coagulability or arterial blood-pressure, and since proteoses prepared from these native proteids, either by means of enzymes or hydrolysis with acids, are toxic, it remained to ascertain whether the proteoses naturally occurring in the vegetable kingdom have any toxic influence when injected.

Several experiments of this order have been carried out with native proteoses obtained from the brazil-nut, hemp-seed, and the wheat embryo.

*Brazil-nut proteose* was prepared as follows: The comminuted nuts were extracted with benzine to remove fat, and then with warm distilled water. The aqueous solution was allowed to stand in the cold for several days, when the excelsin separated. The filtrate was concentrated to a small volume, filtered free from resulting coagulum, and the proteose present precipitated from the filtrate with alcohol.

and poured into a large volume of water containing a little sodium chloride in which the zeïn was insoluble. The hard precipitate was thoroughly washed with distilled water.

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*Hemp-seed proteose* was prepared by extracting hemp-seed at 60° C. with 3 per cent sodium chloride solution from which the edestin separated on cooling. The filtrate, when concentrated by heat and freed from coagulated proteid, was saturated with sodium chloride with the addition of acetic acid. The resulting precipitate of proteoses was dissolved in water and dialyzed free from salt.

*Wheat-embryo proteose* was prepared by extracting wheat germ meal with 3 per cent sodium chloride solution heated to 70° C., dialyzing the extract in water, coagulating the coagulable proteids by heat, and precipitating the proteoses by dialysis in alcohol. This precipitate was dissolved in water, saturated with ammonium sulphate, the resulting precipitate redissolved in water, the solution dialyzed free from sulphate, and then dialyzed into alcohol.

Typical protocols follow.

*Experiment 13.* — Bitch of 5.1 kilos. The animal received an injection (20.4 c.c.) of 1.36 gms. (0.26 gm. per kilo) of *native proteoses*, obtained from the *brazil-nut*,[1] in a faintly acid solution. The injection lasted 40 seconds, during and succeeding which for a short period the animal gave evidences of marked excitation (cries, etc.).

The average clotting time of the blood before the injection was 9 minutes.

Blood samples collected during 30 minutes following the injection had not clotted 12 hours later. Within 20 hours a soft clot had formed. Samples collected 49 min., and 2 hours after the injection, clotted in 4 hours 25 min., and 1 hour 58 min., respectively. The usual fall in blood-pressure was observed.

Chittenden, Mendel, and Henderson[2] have shown that proteoses prepared from typical proteids by enzymes or by hydrolysis with acids may also exercise a lymphagogic influence, at the same time rendering the lymph incoagulable and producing characteristic changes in its composition. A single experiment is submitted here, showing the influence of a *native* proteose upon the blood and lymph.

*Experiment 31.* — A dog of 12 kilos received an injection (48 c.c.) of 6 gms. (0.5 gm. per kilo) of *native proteoses* (obtained from *hemp-seed*), in a weakly alkaline solution. The injection lasted 75 seconds, during which,

[1] The preparation was kindly furnished by Dr. T. B. Osborne of the Connecticut Agricultural Experiment Station.

[2] Chittenden, Mendel, and Henderson: *Loc. cit.*, p. 162.

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and for a short period following which, the animal showed evidences of great excitation (cries, struggles, etc.). This period of excitation was followed by deep narcosis, which culminated in death 2½ hours later.

TABLE SHOWING INFLUENCE UPON LYMPH-FLOW, ETC.

| Time. | Lymph-flow in 10 minutes. | Total solids of lymph. | Ash of lymph. |
|---|---|---|---|
| | c.c. | per cent. | per cent. |
| 10.53–11.03 | 2.3 | 4.00 | 0.87 |
| 11.03–11.13 | 2.1 | | |
| | Injection (see above) | | |
| 11.16–11.26 | 7.2 | 5.78 | 0.71 |
| 11.26–11.36 | 6.7 | 6.33 | 0.85 |
| 11.36–11.46 | 7.0 | | |
| 11.46–12.06 | 3.6 | 5.85 | 0.81 |
| 12.06–12.16 | 2.2 | 5.79 | 0.83 |
| 12.16–12 26 | 3.7 | | |
| 12 26–12 36 | 15.1 | 6.57 | 0.84 |
| 12.36–12.46 | 19.1 | | |
| 12.46–12.56 | 13.0 | | |
| 12.56–1.06 | 8.0 | | |
| 1.06–1.16 | 4.2 | 6.59 | 0.85 |
| 1.16–1.26 | 3.0 | | |
| 1.26–1 36 | 2 5 | | |
| 1.36–1.46 | 0.8 | | |
| 1.46–1.56 | 0.2 | | |
| 1.56–2.06 | 0.8 | | |

There was no change in the mode of respiration to account for the second increased lymph-flow. None of the samples after injection showed the least tendency to clot, and all samples after the injection, with the exception of the first two, were tinged with red.

The average clotting time of the blood before the injection was 15 minutes.

Blood samples collected at intervals during an hour following the injection were still liquid 24 hours later. Samples collected 1 hour

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37 min., and 2 hours 36 min., after the injection, clotted in 2 hours 57 min., and 44 min., respectively. The typical fall in blood-pressure was noted.

The results of these and several similar experiments indicate that in doses of 0.3–0.5 gm. per kilo native proteoses may provoke the effects characteristic of other proteoses.

## Are Products prepared from Edestin with Acid, according to the Methods of Pick and Spiro, truly Non-toxic?

Pick and Spiro have shown that products formed by the action of acid upon fibrin exercise a toxic influence when injected. Products similarly obtained from edestin had no influence either upon the blood-coagulability or upon the blood-pressure. These experiments form the basis for their assertion that the typical effects noted after proteose injection are due to some contamination derived from the animal tissues and absent in the purified vegetable proteids.

In the present investigation the same method of preparing products has been followed.[1]

100 gms. of crystallized edestin were allowed to stand in 2 litres 0.8 per cent hydrochloric acid for one week, at 38° C. The mixture was then filtered, the filtrate neutralized with sodium carbonate, and the resulting precipitate of acid-proteid filtered off. The filtrate was evaporated to dryness on a water bath. The dry residue amounted to 17 gms. of a light brown powder. This material was injected. In the following experiment it was intended to inject 7.25 gms. (1.25 gms. per kilo), but 2 gms. of the material were insoluble.

*Experiment 2.* — (Compare Experiment X, Pick and Spiro[2]). A bitch of 5.8 kilos received an injection (18.8 c.c.) of 5.25 gms. (0 9 gm. per kilo) of the *above product* in a faintly alkaline solution. The injection lasted 35 seconds, during which, and for a short period following which, the animal gave evidence of marked excitation (cries, struggles, etc.). No influence on blood-coagulability was noted. A noticeable fall in pressure was observed.

Four minutes after injection the dog suddenly died. Upon examination it was found that there were clots in the right ventricle and in the large blood-vessels. The intestines were very pale. Analysis of the product injected showed that of the total nitrogen, 14.9 per cent was in the form of compounds precipitable with zinc sulphate.

[1] *Cf.* Pick and Spiro: *Loc. cit.*, p. 252.

[2] Pick and Spiro: *Loc. cit.*, p. 252.

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A second series of experiments was then carried out as follows:

130 gms. of edestin were dissolved in 4½ litres of 0.8 per cent hydrochloric acid, and the solution divided into two equal parts. One portion (Portion I) was allowed to digest one week at 38° C., and then treated as in the previous experiment. It yielded 25 gms. of the final product. The second portion (Portion II) was allowed to digest three weeks, and then treated as above. It yielded 30 gms. Since in the second portion the bulk of the proteid present was precipitated as acid-proteid on neutralization, this precipitate was further digested with 0.8 per cent hydrochloric acid at 100° C. for 12 hours, and then treated as the other portions (= Portion III). The different portions were then injected. Protocols follow:

**Injections of Portion I.** *Experiment 25.* — A dog of 7 kilos received an injection (28 c.c.) of 8.75 gms. (1.25 gms. per kilo) of Portion I in a faintly alkaline solution. The injection lasted 60 seconds. Evidence was obtained of excitation (cries, etc.). No influence on blood-coagulability was observed. There was, however, a noticeable fall in arterial pressure..

Analysis of Portion I showed that of the total nitrogen 87.8 per cent was in a form precipitable with zinc sulphate. A subsequent injection of "Witte's peptone" failed to have any influence upon blood-coagulability; but a distinct fall in blood-pressure was observed.

*Experiment 35.* — A bitch of 6 kilos received an injection (24 c.c.) of 7.5 gms. (1.25 gms. per kilo) of Portion I in a faintly alkaline solution. The injection lasted 30 seconds, and the animal remained quiet.

The average clotting time of the blood before the injection was 14 minutes.

Blood samples collected 1 min., 6 min., 27 min., 50 min., 1 hour 17 min., 1 hour 50 min., after the injection, clotted in 24 min., 42 min., 23 min., 53 min., 28 min., and 41 min., respectively.

The blood-pressure fell very rapidly and remained stationary.

**Injections of Portion II.** *Experiment 30.* — A dog of 6 kilos received an injection (24 c.c.) of 7.5 gms. (1.25 gms. per kilo) of Portion II in a faintly alkaline solution. The injection lasted 30 seconds. There was evidence of great excitation (cries, struggles, etc.), for several minutes, followed by deep narcosis. The average clotting time of the blood before the injection was 17 minutes.

Blood samples collected 2 min., 6 min., 24 min., and 40 min., after the injection, showed no tendency to clot 24 hours later. Samples collected 1 hour 10 min., and 2 hours 6 min., after the injection, clotted in 3

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hours, and 54 min., respectively. The typical fall in the blood-pressure was also obtained.

*Experiment 32.* — A bitch of 9 kilos received an injection (36 c.c.) of 4.5 gms. (0.5 gm. per kilo) of Portion II in a faintly alkaline solution. The injection lasted 43 seconds. The animal remained quiet throughout.

The average clotting time of the blood before the injection was 21 minutes.

Blood samples collected 1 min., 4 min., 6 min., and 56 min., after the injection, clotted in 45 min., 43 min., 58 min., and 28 min., respectively. There was a noticeable fall in the blood-pressure.

Analysis of Portion II showed that of the total nitrogen 51.3 per cent was in a form precipitable with zinc sulphate.

**Injections of Portion III.** *Experiment 41.* — A dog of 7 kilos received an injection (35 c.c.) of 8.75 gms. (1.25 gms. per kilo) of Portion III in a faintly alkaline solution. The injection lasted 35 seconds. There was evidence of great excitation (cries, struggles, etc.), which was followed by deep narcosis.

The average clotting time of the blood before the injection was 21 minutes.

Blood samples collected 1 min., 10 min., 25 min., 48 min., 1 hour 20 min., and 1 hour 40 min., after the injection, clotted in 62 min., 43 min., 25 min., 48 min., 1 hour 38 min., and 45 min., respectively. The typical fall in blood-pressure was also noted.

Analysis of Portion III showed that of the total nitrogen 20 per cent was in a form precipitable with zinc sulphate.

From these experiments it is seen, that although products formed from edestin by a week's hydrolysis with hydrochloric acid may be without influence, yet with products formed by three weeks' hydrolysis the effects noted after injection are typical for the isolated proteoses. The failure of Pick and Spiro to obtain similar results is apparently due to the fact that very little true proteose was contained in their products. In many attempts of the writer to isolate the proteoses in the usual manner by saturation with ammonium sulphate, the effort was fruitless. Little or no proteose was present. It is a matter of extreme difficulty to remove all the acid-albumin-like body from such digestion mixtures, and this is the substance of which their product was probably largely composed. These facts account for the varying proportions of proteid precipitable with zinc sulphate, recorded above, — proportions which in no way correspond to the effects noted; for with Portion I, which contained 87.8 per cent of

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nitrogen precipitable with zinc sulphate, the influence of the injection was very slight, while with Portion II, in which only 51.3 per cent of the total nitrogen was precipitable with zinc sulphate, the effects were very marked. Just what is the nature of this body is unknown, but it is non-coagulable in neutral, and soluble in faintly alkaline, solutions. It may be assumed, therefore, that 0.8 per cent hydrochloric acid acting on edestin for a period of a week is not always sufficient to produce proteoses enough to call forth any noticeable effects after injection; while the products formed by a three weeks' hydrolysis of edestin with the same strength of acid give all the reactions typical of proteoses isolated from other sources.

Inasmuch as excelsin, the brazil-nut proteid, had been shown to be without influence when injected, material was prepared from it by hydrolysis with 0.8 per cent hydrochloric acid at a temperature of 100° C. for a period of twenty hours, and was separated according to the Pick and Spiro method. The product provoked effects of the most pronounced character. Of the total nitrogen of the material 17.6 per cent was precipitable with zinc sulphate. Here no difficulty was experienced in causing a complete precipitation of the acid-proteid on neutralization, and in all probability the nitrogen precipitable by zinc sulphate is a true indication of the quantity of proteoses present.

**Injections of products formed from excelsin with hydrochloric acid.**

*Experiment 45.* — A dog of 10.3 kilos received an injection (41.2 c.c.) of 7.73 gms. (0.75 gm. per kilo) of the *product described above*, in a faintly alkaline solution. The injection lasted 45 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 12 minutes.

Blood samples collected at frequent intervals during 1½ hours following the injection were still liquid 24 hours later. There was a noticeable fall in blood-pressure followed by a gradual rise.

A second experiment, in which a much smaller dose (0.38 mg. per kilo) was given, was also carried out. The effects of this injection were in every way as marked as in the experiment just given.

It has been shown above, that if edestin be treated long enough with hydrochloric acid, there are formed products capable of calling forth effects similar to those following proteose injection. Will proteoses, if treated with acid until there is no longer any body present giving the biuret reaction, yield any substance or substances

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which will induce analogous physiological reactions? The attempt to answer this has been made with proteoses, prepared from edestin by heating with 2 per cent sulphuric acid in a steam sterilizer for nine hours.

10 gms. of the isolated proteoses were boiled with 100 c.c. of 3 per cent hydrochloric acid, for a period of 70 hours, after which no biuret reaction could be obtained. This solution was neutralized exactly with $\frac{n}{10}$ sodium hydroxide, and a portion injected.

*Experiment 36.* — A dog of 8 kilos received an injection of 40 c.c. of the *above solution* corresponding to 4 gms. (0.5 gm. per kilo) of proteoses, in a faintly alkaline solution. The injection lasted 1 minute and 40 seconds. The animal remained quiet. There was no influence upon blood-coagulability, and only a slight fall in arterial pressure, which immediately regained the normal. A second injection (49 c.c.) of 4 gms. (0.5 gm. per kilo) of the undecomposed proteoses to which 40 c.c. of 3 per cent hydrochloric acid were added and the mixture neutralized with $\frac{n}{10}$ sodium hydroxide, followed. The purpose of the addition of the acid and its subsequent neutralization was to make the salt content of the second preparation exactly comparable with that of the first injection. The final reaction of the fluid was faintly alkaline, and the injection lasted 48 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 11 minutes.

Blood samples collected 1 min., 11 min., 28 min., 66 min., and 74 min., after the injection, clotted in 4 hours 41 min., 6 hours 6 min., 5 hours 50 min., 1 hour 22 min., and 54 min., respectively. The typical blood-pressure phenomena were observed.

From these experiments it seems evident that products may be formed according to the method of Pick and Spiro, cited above, which are capable of inducing effects in the organism analogous to those called forth by ordinary proteose injection. We find no occasion to attribute these effects to products other than the proteoses present; and when the latter are split up into simpler compounds the peculiar physiological reactions are no longer obtained.

### Are the Toxic Effects of Proteoses destroyed by the Pick and Spiro Methods of "Purification"?

According to Pick and Spiro, the proteoses lose their toxic effects when they have been treated with either alcohol alone or alcohol and alkalies. In the present research some of the experiments of these

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investigators have been repeated, and the results obtained fail to confirm their observations. The purification of "Witte's peptone" was first carried out.

60 gms. of "Witte's peptone" were dissolved in one litre of equal parts of distilled water and 95 per cent alcohol, and boiled on a water bath under a reflux condenser for 5 hours. After cooling, the insoluble portion was filtered off and dried. The filtrate was evaporated to dryness on a water bath. During the boiling of the substance sulphur derivatives were liberated in considerable quantity. A piece of absorbent cotton moistened with lead acetate and placed in the top of the condensing tube was very readily turned black, owing to the formation of lead sulphide.

**Influence of the insoluble portion.** *Experiment 34.* — (Compare Pick and Spiro, Experiment XVIII[1]). A dog of 10.5 kilos received an injection (42 c.c.) of 5.25 gms. (0.5 gm. per kilo) of the *insoluble portion* of "Witte's peptone" treated as above, in a faintly alkaline solution. The injection lasted 30 seconds. The animal remained quiet, but 52 minutes after injection suddenly died.

The average clotting time of the blood before the injection was 16 minutes.

Blood samples collected at frequent intervals after the injection showed no tendency to clot 24 hours later. The blood-pressure fell from 105 mm. Hg to 9 mm., where it remained until the animal's death.

**Influence of the soluble portion.** *Experiment 37.* — (Compare Pick and Spiro, Experiment XIX[2]). A bitch of 10 kilos received an injection (40 c.c.) of 5 gms. (0.5 gm. per kilo) of the *soluble portion* of "Witte's peptone" treated as above, in a faintly alkaline solution. The injection lasted 30 seconds. There was evidence of great excitation (cries, etc.), followed by deep narcosis.

The average clotting time of the blood before the injection was 17 minutes.

Blood samples collected 1 min., 7 min., 21 min., and 23 min., after the injection, had not clotted 24 hours later. The typical blood-pressure phenomena were also observed.

The next experiment corresponds with Experiment XX[3] of Pick and Spiro, in which "Witte's peptone" was injected after being boiled ten hours with alcohol.

A litre of a 6 per cent solution (equal parts distilled water and 95 per cent alcohol) was boiled on a water bath, under a reflux condenser for 10

[1] *Loc. cit*, p. 256. [2] *Loc. cit.*, p. 257. [3] *Loc. cit.*, p. 257.

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hours. At the end of this period the mixture was evaporated to dryness upon the water bath. In this experiment sulphur compounds were also liberated in considerable quantity during the boiling.

*Experiment 38.* — A dog of 9.2 kilos received an injection (36.8 c.c.) of 3.4 gms. (0.38 gm. per kilo) of "Witte's peptone" treated 10 hours with alcohol, in a faintly alkaline solution. There was evidence of marked excitation (cries etc.), which was followed by deep narcosis. The injection lasted 30 seconds.

The average clotting time of the blood before the injection was 14 minutes.

Blood samples collected 2 min., 7 min., 32 min., 58 min., 1 hour 27 min., and 1 hour 41 min., after the injection, clotted in 7 hours, 1 hour, 36 min., 30 min., 17 min., and 15 min., respectively. The usual fall in blood-pressure was observed.

*Experiment 39.* — A dog of 8 kilos received an injection (32 c.c.) of 4 gms. (0.5 gm. per kilo) of the *above product*, in a faintly alkaline solution. The injection lasted 30 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 9 minutes.

Blood samples collected at frequent intervals for 1½ hours after the injection showed no tendency to clot 24 hours later.

The blood-pressure fell from 140 mm. Hg to 24 mm., where it remained.

From the preceding it appears that treatment of "Witte's peptone" with alcohol, according to the methods of Pick and Spiro, has little or no detrimental influence on the active agent. In the experiment (Experiment 38), where the toxic action of "Witte's peptone" seemed to be decreased somewhat after treatment with alcohol, may not the fact that some decomposition of the products had taken place, as indicated by the liberation of sulphur derivatives, suffice to account for the lessened toxicity of the substance?

In a further series of experiments, protoproteose was separated from heteroproteose in the manner described by Pick and Spiro.[1] The original product was obtained from a peptic digestion of edestin. The separation of the two primary proteoses from each other was effected as follows: 10 grams of the mixture were dissolved in 120 c.c. of water, to which were added 2 volumes of 95 per cent alcohol, and the whole boiled thirteen hours on a water bath, under

[1] *Loc. cit.*, p. 255. See also PICK: Zeitschrift für physiologische Chemie, 1898, xxviii, p. 219.

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a reflux condenser. The mixture was allowed to cool, when a flocculent precipitate (heteroproteose) separated. This was dried. The filtrate (protoproteose) was evaporated to dryness on the water bath. About 5 grams of each were obtained.

**Injection of protoproteose.** *Experiment 40.* — (Compare Experiment XV, Pick and Spiro[1]). A dog of 8.3 kilos received an injection (33.2 c.c.) of 4.15 gms. (0.5 gm. per kilo) of *protoproteose*, in an alkaline solution. The injection lasted 30 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 15 minutes.

Blood samples collected at frequent intervals during the first hour after the injection had not clotted 24 hours later. Samples collected during the second hour after the injection clotted in 8 hours. The typical phenomena connected with the blood-pressure were observed.

**Injection of heteroproteose.** *Experiment 43.* — (Compare Experiment XVI, Pick and Spiro.[2]) A dog of 9.5 kilos received an injection (38 c.c.) of 4.75 gms. (0.5 gm. per kilo) of *heteroproteose*, in a faintly alkaline solution. The injection lasted 30 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 10 minutes.

Blood samples collected 2 min., 4 min., 29 min., 54 min., and 1 hour 27 min., after the injection, clotted in 38 min., 47 min., 36 min., 31 min., and 22 min., respectively. The fall in blood-pressure was typical.

A third method[3] of purification as given by Pick and Spiro was also repeated in the present investigation. According to these authors, proteoses subjected to treatment with an alkaline alcohol solution for twenty-four hours lose their toxic effects.

The proteoses employed in this experiment were "hemialbumoses," already referred to under Experiment 18, page 355. They were treated as follows: 20 grams of hemialbumoses were dissolved in 200 c.c. of water, and 2 volumes of 95 per cent alcohol added. The mixture was made faintly alkaline with sodium carbonate, and boiled for 24 hours on a water bath, under a reflux condenser. At the end of this time the solution was cooled, and a small precipitate which separated was filtered off. The filtrate was evaporated to dryness on the water bath, and injected. The precipitate noted above was too small for an injection.

**Injection of "purified" hemialbumose.** *Experiment 20.* — A dog of 6 kilos received an injection (24 c.c.) of 3 gms. (0.5 gm. per kilo) of "*purified*"

[1] *Loc. cit.*, p. 255. [2] *Loc. cit.*, p. 255. [3] *Loc. cit.*, p. 259.

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*hemialbumose*, in a faintly alkaline solution. The injection lasted 55 seconds. During the injection and for a short period following there was evidence of great excitation (cries, etc.), and this period of agitation was succeeded by deep narcosis. Evidence was also obtained of increased intestinal peristalsis.

The average clotting time of the blood before the injection was 13 minutes.

Blood samples collected 2 min. after the injection clotted in 6 hours 25 min. Samples collected 6 min. and 34 min. after the injection clotted within 20 hours. Samples collected 2 hours 7 min., and 2 hours 54 min., after the injection, clotted in 62 min., and 24 min., respectively.

The blood-pressure fell from 118 mm. Hg to 30 mm., and 2 hours later had risen to 70 mm., where it remained.

Other proteoses, obtained by peptic digestion of egg-yolk vitellin, and separated in the manner usual in this investigation, were likewise subjected to treatment with an alkaline alcohol solution. In this case considerable sulphide was set free. Practically all of the material was soluble. A protocol follows:

**Injection of "purified" vitelloses.** *Experiment 23.* — A dog of 9.8 kilos received an injection (39.2 c.c.) of 4.9 gms. (0.5 gm. per kilo) of the *above product*, in a faintly alkaline solution. The injection lasted 45 seconds. There was evidence of great excitation (cries, etc.), which was followed by deep narcosis. Defæcation occurred.

The average clotting time of the blood before the injection was 10 minutes.

Blood samples collected at frequent intervals during 2 hours following the injection showed no tendency to clot 24 hours later.

The blood-pressure fell from 108 mm. Hg to 28 mm., and at the end of 2 hours had risen to 71 mm.

The results of the experiments here recorded justify the conclusion that the methods of purification adopted by Pick and Spiro are of questionable value, since the products so treated lose scarcely any of their toxic properties.

In an article by Spiro and Ellinger[1] the statement is made that "somatose" is an albumose made without the use of enzymes, and when injected is without influence on blood-coagulability.

The proteoses separated from such a preparation (Friedr. Bayer & Co., Elberfeld, Germany), by precipitation with ammonium sulphate

[1] *Loc. cit.*, p. 158.

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and dialyzed free from salt, provoked the effects typical for proteoses. The proctocol follows :

**Injection of somatose.** *Experiment 28.* — A dog of 7.9 kilos received an injection (31.6 c.c.) of 3.95 gms. (0.5 gm. per kilo) of *proteoses* separated from *somatose*, in a neutral solution. The injection lasted 34 seconds. The animal remained quiet.

The average clotting time of the blood before the injection was 18 minutes.

Blood samples collected at frequent intervals during 40 min. after the injection had not clotted in 8 hours, but within 20 hours were found clotted. Samples collected 53 min. after the injection clotted in 51 min.

The blood-pressure fell from 125 mm. Hg to 70 mm., and within 4 min. had risen to 100 mm., where it remained.

## Conclusions.

A review of the experimental work presented in this paper leads us to the conclusion that there is at present no occasion for attributing the physiological effects following the injection of proteoses into the circulation to the presence of contaminating substances derived from animal tissue or elsewhere. Typical purified vegetable proteids which when injected are themselves inert in this regard, yield on hydrolysis with acids, or even water alone, proteoses which provoke the characteristic reactions. The proteoses which are formed by the action of proteolytic enzymes of vegetable origin (bromelin, papaïn) on purified proteids, likewise alter *in vivo* the coagulability of the blood and call forth the other well-known symptoms of proteose injections. The proteoses occurring in nature in the vegetable kingdom are similarly active, as was to be expected if the toxic properties are a function of these products *per se*. No method of "purification" has been found which will deprive proteoses of this characteristic physiological behavior in the circulation; when the chemical make-up of the proteoses is profoundly altered and they lose their chemical identity, the typical physiological action may also be lost.

We regard the failure of other investigators to obtain toxic products by hydrolysis of proteids with acids, etc., as attributable, in part at least, to the fact that they injected mixtures of unknown composition and probably containing either insufficient doses of proteoses proper, or antagonistic compounds simultaneously present. It has, in fact,

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been demonstrated above that it is possible to prepare, by methods with which Pick and Spiro failed to obtain positive results, products which will provoke effects analogous to those obtained with isolated proteoses. Incidentally we have again learned the differences in the susceptibility of different animal species to the toxic action of injected proteoses, by comparing the response in the dog with that in the cat. Recent studies on immunity have shown equally striking natural differences among animals, and have again emphasized the influence which the mode of introduction of toxins may exert. Finally, there is at present no reason for denying the existence of many chemically unrelated substances which call forth apparently similar responses in the animal organism.

This investigation has been carried out at the suggestion of Professor Lafayette B. Mendel, to whom the writer desires to express his obligation for help and criticism. The expenses of the research were defrayed by an appropriation from the Rockefeller Institute for Medical Research.

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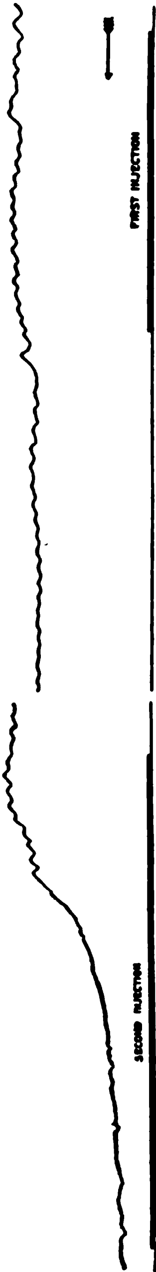
TYPICAL BLOOD-PRESSURE TRACINGS SELECTED FROM THE PRECEDING EXPERIMENTS.




Experiment 18. — Injection of crystallized egg-albumin; subsequent injection of "hemialbumoses." (See page 355.)

Experiment 31. — Injection of native proteose from hemp-seed. (See page 360.)

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# ON RIGOR MORTIS.

By OTTO FOLIN.

[*From the Chemical Laboratory of the United States Fish Commission, Woods Hole, and the Chemical Laboratory of the McLean Hospital for the Insane, Waverley, Mass.*][1]

KÜHNE is frequently credited with the authorship of the current theory that rigor mortis is the effect of spontaneous coagulation of certain proteins of muscle plasma after death. This is incorrect. The coagulation theory of rigor mortis was advanced, and in fact generally accepted, several years before Kühne took up the subject. Johannes Müller,[2] for example, discussed the possibility of muscle rigor being due to fibrin formation of lymph and blood as far back as 1837. Müller does not commit himself to the theory, to be sure, but he clearly favors it as against the earlier "contraction" theory. Five years later Brücke[3] defended the view that rigor mortis is due to the coagulation of a substance in the muscle plasma which he conceives to be more or less identical with the fibrinogen of blood plasma. It is undoubtedly largely due to this paper of Brücke's that the coagulation theory came to be the most generally accepted view.

In 1858, one year before the publication of Kühne's first paper on the subject, Ludwig wrote in his Lehrbuch der Physiologie des Menschen, p. 472, as follows: "There is now scarcely any difference of opinion in regard to whether muscle rigor is due to the coagulation of a liquid protein substance, while there is every variety of opinion in regard to the exact nature of that substance. On the same page Ludwig writes: "The objection which has been raised against the coagulation theory, that no one has been able to press out of fresh muscles the liquid fibrin substance the coagulation of which produces rigor, is not well taken, as Brücke points out, because while

[1] The investigations which form the basis of this paper were begun in Woods Hole during the summer of 1902, and I wish here to express my obligation to the U. S. Fish Commission for the use of the laboratories, and for other conveniences which were freely extended to me during my stay at Woods Hole.

[2] MÜLLER: Handbuch der Physiologie des Menschen, 1837, ii, p. 46.

[3] BRÜCKE: Archiv für Anatomie und Physiologie, 1842, p. 179.



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the muscles are being subjected to the pressure necessary to accomplish such a purpose they go into rigor."

It was at this point that Kühne[1] obtained from fresh frog muscles a muscle plasma which possessed all the properties that had been demanded by the opponents of the coagulation theory. This plasma not only coagulated at 40°, the temperature at which frog muscles go into rigor, but, like blood plasma, it coagulated spontaneously at ordinary room temperature. By proving the presence in fresh muscles of such a coagulable protein and by showing that it is no longer to be found in muscles which have gone into normal rigor, Kühne silenced all opposition to the coagulation theory of rigor.

Subsequent investigators, Halliburton, Fürth,[2] G. N. Stewart and Sollman,[3] and a very large number of other workers, have brought out many interesting and important points in regard to the coagulation of the proteins of muscle plasma and the conditions governing rigor. The fundamental proposition that rigor mortis is due to the coagulation of certain muscle proteins remains, however, much as Kühne left it. And his statement (*loc. cit.*, p. 773) that "rigor mortis is a coagulation, and all other opinions in regard to it are nothing but pure speculations because there is not a single reason for assuming that any other process than coagulation is involved in its production," may still be considered practically unchallenged.

While the coagulation theory may thus be considered as well nigh universally accepted it can scarcely be said that it has gained in strength or clearness since it was first advanced as an explanation of the phenomenon of rigor mortis. The admitted fact that muscle rigidity may begin before any visible coagulation of the plasma has occurred seems strangely inconsistent with the idea that coagulation is the cause of the rigidity. The fact that the onset of rigidity may be prevented and rigidity already produced may be made to disappear by simply gently bending or pressing a muscle between the fingers would seem far more in harmony with a "contraction theory" than with the coagulation theory of muscle rigor. The disappearance of rigor which by the above theory should be due to a redissolution of the coagulated "myosin fibrin" inside the muscle fibres has become, as Stewart and Sollman (*loc. cit.*, p. 456) truly observe, "more mysterious than ever" since recent investigations have com-

[1] KÜHNE: Archiv für Anatomie und Physiologie, 1859, p. 788.
[2] FÜRTH: Beiträge zur chemischen Physiologie und Pathologie, 1903, iii, p. 543.
[3] STEWART and SOLLMAN: Journal of physiology, 1899, xxiv, p. 427.

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pletely failed to find any adequate explanation of this part of the phenomenon.[1]

The experiments to be described in this paper were undertaken for the purpose of showing that the coagulation theory of rigor is incorrect. The investigation was begun with fishes at Woods Hole, but the facts here stated refer exclusively to later experiments made with frogs' muscles, since the original experiments of Kühne which have been taken to prove the coagulation theory were made with that material.

Many physiologists, especially the writers of text-books, seem at present to include in the term "rigor mortis" several different processes, such as the evolution of carbon dioxide, of acids and of heat, the shortening of muscles and their becoming "cloudy" or opaque. As far as I am aware, however, it has never been shown that any of these phenomena except the last stand in any causal relationship to the fundamental phenomenon of rigidity and hardness or to the coagulation process which is supposed to produce the latter. The term "rigor" is therefore here taken in a more restricted sense, *i. e.* as representing only that peculiar stiffening and hardening of muscles occurring during or after cessation of life.

To prove that rigor mortis in this sense is not a coagulation phenomenon I produce rigor in frog muscles by subjecting them to a temperature of $-15°$ to $-20°$ C.

The fact that fresh muscles can be made to go into rigor by simply lowering their temperature does not seem to be generally known. It is, however, no new discovery. In 1842 Brücke (*loc. cit.*, p. 186) described rigor produced by cold and maintained that the rigor so obtained is in every way identical with normal rigor. Brücke's experiments with cold as a means of producing rigor received, curiously enough, no attention from subsequent investigators, and as far as I have been able to learn the discovery has been forgotten.

Rigor mortis obtained in this way appears, however, much better adapted to throw light upon the process that gives rise to it than is "normal" rigor or heat rigor. In cold rigor we seem in fact to have the fundamental phenomenon of rigor mortis isolated, so to speak,

[1] On this point see especially FÜRTH's last paper (*loc. cit.*, p. 549). FÜRTH shows that the disappearance of the "muscle clot" is not accounted for by the increased acidity of the muscle serum, nor can he find the least evidence for HALLIBURTON's view that this end is brought about by means of some ferment reaction.

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from those other processes which are usually associated with the onset of rigidity. That this should be so is not to be wondered at, considering under what conditions cold rigor occurs in frog muscles.

The muscles of frogs can be cooled to $-7°$ C., *i.e.* they can be frozen solid without losing their irritability and contractility when carefully thawed. If the cooling of such frozen muscles is continued longer and their temperature lowered to $-15°$, the power to recover contractility is lost, and on thawing such muscles they will be found to have gone into rigor. It seems clear that the chemical changes involved in the transformation of the frozen but potentially living muscle into the dead and rigid muscle must be relatively very small. Such low temperatures and the absence of a liquid medium acting together would tend to reduce to a standstill all those chemical reactions which are associated with constructive or destructive metabolism, *i.e.* oxidations, reductions and ferment or other hydrolytic reactions.

Considering these conditions, it seems indeed remarkable that rigor mortis should be produced by lowering the temperature of the muscles from $-7°$ C. to $-15°$ C., but it is not surprising that few if any other changes occur, and that the rigor so produced should appear to be a more or less isolated phenomenon. Such is the case. The rigid muscles are apparently not shortened, they are still neutral or faintly alkaline to litmus, and they show absolutely no sign of cloudiness or opaqueness which could point to coagulation as the cause of the rigidity.

My experience so far indicates that it is not possible to freeze frog muscles to a point where they have not acquired the rigidity of rigor mortis and yet have lost the power of recovering contractility on thawing. Should further experiments verify this observation it would indicate that in this case the two occurrences — loss of contractility and onset of rigor — may be more or less directly due to the same cause. Later I hope to make further attempts toward studying this phase of the problem. The investigations here described are directed exclusively toward proving that the rigidity and hardness of muscles in rigor are not due to a coagulation process.

The perfect translucency of muscles which have gone into cold rigor as compared with fresh living muscles would itself seem to disprove the theory that this phenomenon is due to a coagulation process, or it would at least force one to the unwarranted assumption that the coagulum itself must be translucent. Muscles of this kind

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kept at ordinary room temperature remain both rigid and translucent until they begin to decompose.

I have, however, not relied upon this transparency alone, but have made quantitative tests as follows:

All the muscles from the hind legs of two frogs are removed and divided into two equal portions. Each portion is then placed on a filter paper or on a watch glass in the bottom of a tin can, and to each portion is added one gastrocnemius muscle as control. One tin can is placed on ice for two or three hours. The other is immersed in a freezing mixture for the same length of time.

The frozen muscles are taken out first, and the control gastrocnemius is laid aside to thaw in order to show that rigor has actually taken place. The rest of the frozen muscles are macerated in a cold mortar and thoroughly rubbed up with 25 c.c. cold sodium chloride solution (0.7 per cent). The resulting mixture is filtered and the residue rubbed up with another 25 c.c. salt solution. The second filtrate is added to the first.

The other tin can containing muscles kept on ice only is next placed on the top of the freezing mixture and watched until the muscles begin to solidify. They are then taken out at once and macerated and extracted just as in the case of the frozen muscles. The control gastrocnemius in this case will show that no rigor has taken place. (The same results can be obtained without subjecting the contents of the second tin can to any freezing at all, but it is then much more difficult to macerate the muscles completely.)

All this work was done in my experiments in a large ice-room which was further cooled by liquid ammonia pipes, but such precautions are not at all necessary for the success of the experiment, because fresh muscle plasma does not coagulate spontaneously with any great degree of rapidity.

It is clear that the two saline solutions obtained above should be quite different if the rigor in the one set of muscles had been produced by the coagulation of dissolved protein. The two are, however, absolutely identical except for the fact that the plasma solution obtained from the frozen muscles is usually a trifle clearer than the other. The solutions are tested as follows:

(*a*) Samples of each are set aside in test tubes at room temperature; both show the next day about the same amount of coagulum.

(*b*) Other samples of each are carefully heated side by side in test tubes immersed in water. The contents of both tubes coagulate

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at the same time at 40°–42° C. and the amount of coagulum is so nearly alike in quantity as to make it impossible to distinguish one from the other.

(*c*) 10 c.c. of each is titrated with tenth normal sodic hydrate in the presence of phenolphtalein. Both show the same degree of acidity, usually amounting to 0.4–0.6 c.c. $\frac{n}{10}$ acid (which means neutral or alkaline reaction to litmus).

(*d*) Finally the total nitrogen is determined in 5 c.c. of each extract, and is found to be identical in both cases, thus excluding absolutely the possibility of one extract containing any more protein than the other.

The above experiments have been repeatedly performed, and always with the same results. They prove, it seems to me, that muscle rigor is independent of the protein coagulation which is usually observed when muscles go into normal rigor, for since muscles can be made to acquire the stiffness and hardness characteristic of rigor mortis without the least indication of any coagulation of protein having occurred, there is clearly no substantial reason for assuming that those same characteristics are dependent on protein coagulation in the case of normal rigor.

The coagulation theory of rigor seems to have originated in a rather superficial analogy between the coagulation of blood and the hardening of muscles. The only other evidence in its favor is the opaqueness which usually accompanies the rigidity, and the experiments of Kühne showing that the muscle plasma obtained from muscles which have gone into rigor is different from the plasma obtained from fresh muscles in that it does not contain the mother substance of myosin fibrin the coagulation of which is supposed to be the cause of the rigor. It is clear that this evidence in favor of the coagulation theory loses all its force in view of the observations recorded in this paper, and in view of the fact that the above positive findings of Kühne might readily be due to other chemical changes occurring in frog muscles during the 40–50 hours that one must wait for them to go into normal rigor.

It would seem that the method of producing rigor by means of cold would offer a fruitful field for further study, and I mean to continue the work from the point of view recorded in this paper.

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# ON THE FORMATION OF DEXTROSE IN METABOLISM FROM THE END-PRODUCTS OF A PANCREATIC DIGEST OF MEAT.

By PERCY G. STILES and GRAHAM LUSK.

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## INTRODUCTORY.

THE key to the chemistry of the proteid molecule has been sought not only in the behavior of proteid in the beaker and test-tube, but also through observations on the metabolism of proteid in plant and animal life.

An extended review of the literature concerned in our problem is manifestly unnecessary, because of the completeness of Langstein's article, " Die Bildung von Kohlehydraten aus Eiweiss,"[1] of Cremer's article on glycogen,[2] and of Pflüger's[3] more recent monograph upon the same subject. Brief mention of important points only can be given in this place.

Kühne and Chittenden believed that the result of pancreatic proteolysis was a production in equal amounts of hemi- and antipeptone, of which the hemipeptone only was further resolved into amido acids.

Kutscher[4] showed that prolonged tryptic digestion completely changed proteid into a mixture of amido acids such as may be obtained by boiling proteid with acids. Cohnheim[5] discovered erepsin, a ferment which rapidly splits albumoses into amido bodies within the intestines; and Loewi,[6] after feeding a pancreatic digest consisting of amido bodies, has been able to maintain a dog in nitrogen equilibrium almost as well as after feeding proteid. Loewi concludes that he has

[1] LANGSTEIN: Ergebnisse der Physiologie, 1902, i, p. 63.

[2] CREMER: *Ibid.*, p. 803.

[3] PFLÜGER: Archiv für die gesammte Physiologie, 1903, lxxix, p. 1.

[4] KUTSCHER: Die Endprodukte der Trypsinverdauung, Strassburg, 1899.

[5] COHNHEIM: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 451.

[6] LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 303.



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proved that a proteid synthesis from amido bodies takes place within the organism.

The fact that various proteids yield amido bodies different in kind and amount has led Emil Fischer to make a prolonged series of investigations in this direction. Kossel[1] first drew attention to the fact that cleavage products of proteid, such as leucin, histidin, lysatinin, lysin, and arginin, each contained the same number of carbon atoms as dextrose, and compared an aggregation of amido acid radicles forming proteid, with the analogous polysaccharides. At the same time Kossel, in conversation with one of the present writers (L.), declared his belief that these amido bodies are the mother substances of the proteid dextrose found in diabetes. This idea was later advanced in a paper by F. Müller,[2] who stated that if proteid could yield 50 per cent of leucin, as was found by Cohn, it could hardly contain a sugar radical equal to 60 per cent, which was the amount of dextrose formed from proteid in phlorhizin diabetes as determined by Reilly, Nolan, and Lusk.[3]

Lusk[4] at this time suggested that leucin, an amido fatty acid, might arise from dextrose molecules in the proteid complex. This view was discussed by F. Müller,[5] who considered it improbable, although intimating that it was difficult to disprove.

The proteid of meat and gelatine yield 60 per cent of dextrose in diabetic metabolism,[6] and Halsey[7] has shown the same to be true of casein. But Halsey could not show that feeding leucin increased the dextrose excretion in phlorhizin diabetes. Cohn[8] believed that the liver glycogen was increased in rabbits after feeding leucin, but these experiments have never been considered conclusive. Recently Stookey[9] has fed various gluco-proteids to fasting hens and has observed little glycogen formation in consequence.

At the time this research was commenced the problem offered was: what amount of dextrose, if any, would be produced after feeding a

[1] KOSSEL: Deutsche medicinische Wochenschrift, 1898, p. 58.

[2] MÜLLER and SEEMAN: Deutsche medicinische Wochenschrift, 1899, p. 209.

[3] REILLY, NOLAN, and LUSK: This journal, 1898, i, p. 395.

[4] LUSK: This journal, 1899, iii, p. 153.

[5] MÜLLER: Zeitschrift für Biologie, 1901, xlii, p. 545.

[6] REILLY, NOLAN, and LUSK: *Loc. cit.*

[7] HALSEY: Sitzungsberichte der Gesellschaft zur Beförderung der gesammten Naturwissenschaften, Marburg, 1899, p. 102.

[8] COHN: Zeitschrift für physiologische Chemie, 1899, xxviii, p. 211.

[9] STOOKEY: This journal, 1903, ix, p. 138.

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phlorhizinized dog with a digestive mixture of proteid decomposition products? If a mixture of amido bodies yields dextrose, then the synthetic origin of dextrose in metabolism as advocated by Pflüger, F. Müller, Halsey, and others, would be established, whether such sugar arose directly from the amido bodies fed or indirectly from the metabolism of proteid formed from them.

After this research was well under way, Knopf,[1] using the same method, published an account showing the synthetic production of sugar from asparagin fed to a dog.

## Method.

In order to measure the production of sugar, phlorhizin diabetes was induced. After one or two days of fasting phlorhizin was administered three times daily by hypodermic injections. The amount given each time was 2 gms. dissolved in about 20 c.c. of 1.2 per cent sodium carbonate solution. The injections were given at approximately equal intervals. On the third day of phlorhizin treatment (the fourth or fifth of fasting) the metabolism usually reaches a level which will be maintained with remarkable uniformity through several subsequent days, the nitrogen elimination being very nearly constant and the sugar appearing in quantity according to a ratio which does not vary widely from that previously determined by Lusk $\left(\frac{D}{N} = 3.75\right)$. The even conditions of phlorhizin diabetes gave us a base-level above which the production of additional sugar from the pancreatic digest fed stood out conspicuously.

**The digest.**[2] — This was obtained by the pancreatic digestion of washed meat which had been allowed to continue for fourteen months with proper precautions against putrefaction. The resulting mixture of products was a dark, syrupy fluid with but little sediment or suspended matter. The taste and odor were pronounced but not foul. The mixture gave only a dubious biuret reaction. As prepared for our use, the material contained 1.33 per cent of nitrogen and gave a depression of the freezing point of 1.72° C. This had a practical interest, for the concentration of salts in such digests must have much to do with the possibility of retaining them in the stomach. If they

[1] Knopf: Zeitschrift für physiologische Chemie, 1903, xlix, p. 123.

[2] This was kindly furnished us by Prof. W. J. Gies of Columbia University, to whom we express our thanks.

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are too concentrated they are sure to be vomited; if they are too dilute, excessive volumes must be given. It is difficult to hit upon a desirable mean. It was always necessary to give the fluid by a stomach-tube, as none of the dogs would take it voluntarily.

**Difficulties.** — Only two of five experiments ran a successful course. In two cases failure of the kidneys occurred, and death followed closely upon the suppression of the urine. In another instance stubborn vomiting compelled us to give up the trial. Fortunately the two experiments which were carried through without mishap were closely concordant.

## Experiments.

*Experiment I.* — The animal was a bitch weighing about 16 kg. On the third day of diabetes the urine was analyzed and the sugar was found to be high in proportion to the nitrogen, the ratio of 4.15 indicating that the preliminary sweeping of sugar from the system had not been accomplished. The urine of the following night gave a ratio of 3.94, and it was considered that the diabetic condition had become sufficiently settled to attempt the feeding of the digest. Just before this was done a final sample of the urine from the bladder gave the D : N ratio of 3.66. The first portion of the digest introduced amounted to 200 c.c. (N, 2.66 gms.). After an hour and fifteen minutes the dog vomited 150 c.c. of fluid, which was saved, and an hour later returned to the stomach together with 200 c.c. of the original mixture. The dog had now received 5.32 gms. of nitrogen in the form of simple digestive products. There was no more vomiting for four hours, when about 250 c.c. of fluid, only slightly colored, was ejected. This was not returned, but analyzed for nitrogen, and found to contain but 0.18 gm. The quantity of nitrogen fed and retained was estimated at 5.14 gms. The stomach remained irritable, and there was vomiting during the following night, but apparently only of water and mucus. The dog defæcated once in the middle of the feeding period; the fæces were moderate in amount and did not differ from those passed during fasting. The analyses of the urine are tabulated on page 384.

*Experiment II.* — The animal was a large bitch weighing 32.6 kg. After three days of diabetes and six of fasting the urine was collected and analyzed for two preliminary periods of twelve hours each. At the beginning of the third period, 400 c.c. of the digest (N, 5.32 gms.) was given and retained without any subsequent sign of digestive disturbance or any diarrhœa. The urine of the feeding period and the twelve hours following was analyzed. The findings are given on page 384.

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EXPERIMENT I.

| | N. | D. | $\frac{D}{N}$. | N fed. |
|---|---|---|---|---|
| I. Preliminary 12 hours [1] . | 7.04 | 27.78 | 3.94 [2] | |
| II. Feeding period 12 hours | 11.38 | 39.58 | 3.48 | 5.14 |
| III. After period 12 hours . | 8.22 | 28.00 | 3.40 | |

[1] The analyses in the preliminary stage were actually for a period of sixteen hours. The figures have been reduced to a twelve-hour basis.
[2] Ratio in last sample of bladder urine = 3.66 : 1.

EXPERIMENT II.

| | N. | D. | $\frac{D}{N}$. | N fed. |
|---|---|---|---|---|
| First preliminary 12 hours . . | 11.31 | 44.54 | 3.94 | |
| Second preliminary 12 hours . | 10.70 | 41.58 | 3.88 | |
| Feeding period 12 hours . . | 14.63 | 54.20 | 3.72 | 5.32 |
| After period 12 hours . . . | 12.35 | 40.71 | 3.29 | |

## Discussion of the Tables.

In both cases the nitrogen fed seems to have been quantitatively eliminated. In Experiment I the excess of nitrogen excreted in the feeding period as compared with fasting is 4.34 gms., and in the after period the excess is 1.18 gms., a total of 5.52 gms. nitrogen excreted for 5.14 gms. fed. In Experiment II the excess of nitrogen elimination in the feeding period over the preceding twelve hours is 3.93 gms., and in the after period 1.65 gms., a total of 5.58 gms. nitrogen excreted for 5.32 gms. fed.

In both experiments the sugar rose in the feeding period, in Experiment I by 11.80 gms., in Experiment II by 12.62 gms. It is noteworthy that the excess of nitrogen elimination extends over two periods, while that of sugar is limited to one. This circumstance accounts for the low D : N ratios in the after periods. The sugar had fallen to the fasting level, while the nitrogen remained markedly above it. If

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all the excess of nitrogen had appeared in the feeding-period, we should have had ratios for that period of 3.10 and 3.33 respectively, and a return in the final period to values of 3.97 and 3.80 in the two experiments. It may be that a return to a higher ratio could have been demonstrated if the urine had been collected during an additional twelve hours. It is to be regretted that the desirability of doing this was not recognized at the time.

## Conclusions.

It appears that 5 gms. nitrogen fed in the form of the products of pancreatic digestion may give rise to the formation of about 12 gms. of dextrose, or D : N : : 2.4 : 1. The same amount of nitrogen fed as native proteid would be expected to produce 18 to 19 gms. of sugar. No light is thrown upon the question whether the sugar in our experiments was formed after a proteid synthesis had occurred or more directly from the amido-bodies. Neither have we any evidence as to the relative importance of the several digestive products which were fed. Further trials, made with the individual bodies present in such mixtures and in pursuance of Knopf's plan as applied to asparagin, may clear up this matter. The experiment shows that it is impossible for a large sugar radical to exist in the proteid molecule. It will be noticed that the amido nitrogen fed was quantitatively eliminated, and did not protect the body's proteid as do meat and gelatine under similar circumstances. It is interesting to possess new facts which show how closely parallel is the course of the sugar metabolism after feeding amido-bodies with that which follows upon a proteid diet.

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# ON THE QUESTION OF PROTEID SYNTHESIS IN THE ANIMAL BODY.

By YANDELL HENDERSON AND ARTHUR L. DEAN.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THAT the animal body is endowed with very limited capacities for synthetic processes is a current and habitual assumption rather than a principle demonstrated by experiment. For while the corollary of this statement — that plants are solely devoted to synthesis — has been found insufficient by botanists, physiologists, mainly perhaps because of the experimental difficulties involved, have continued to accept the dictum of Liebig so far as it applies to animals with little modification, and until recently with no serious question.

The recent discovery by Cohnheim [1] of the ferment "erepsin" is full of suggestion. Cohnheim's investigations show that in the well-known experiment of Neumeister — the supposed reconversion of albumose to coagulable proteid by the action of intestinal mucosa — the primary products of digestion are in fact further decomposed into the ultimate products of proteolysis — into simple crystalline nitrogenous substances. If such an alteration take place to any considerable extent in the normal course of absorption, it is evident that within the animal body there must be a mechanism capable of resynthesizing these simple substances to form the proteids of the blood and tissues.

The question thus raised has been put to the direct test of experiment by Loewi. In the preliminary report of his experiments Loewi stated that he had succeeded in maintaining a dog in nitrogenous equilibrium on a diet in which all albuminous material had been replaced by the products of the prolonged self-digestion of pancreas. In the account of his completed investigations published recently, Loewi [2] gives the details of repeated experiments all yielding a similar result. This is the more striking because it was accomplished in spite of the

[1] COHNHEIM: Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 451.

[2] LOEWI: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 303.



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disturbances of the alimentary tract which ordinarily occur on a diet of predissolved material. So successfully was this difficulty overcome in one of the later experiments that the animal exhibited a gain in weight and a plus balance between the income and output of nitrogen. Loewi interprets this retention of nitrogen as indicating that the proteid cleavage products of the diet were resynthesized to supply the needs of the tissues for proteid. The importance of the subject seemed to warrant an immediate test of the validity of this view. For this purpose — rather than as a mere repetition of Loewi's experiments — we have employed in the experiment detailed below not the products of pancreatic digestion, but those resulting from the action of a mineral acid on proteid by prolonged boiling.

Four kilos of lean beef were carefully freed from fat and connective tissue and run through the meat-chopper. This material was placed in a flask with two litres of water containing 240 c.c. of concentrated sulphuric acid. The flask was heated in a steam sterilizer for twenty hours, and then upon a sand bath, where its contents were boiled for two hundred and fifty hours. The volume was kept at about five litres, so that the strength of acid was from seven to nine per cent. Repeated tests made during the boiling showed that a biuret reacting substance was continually being split off in small amounts from the antialbumid which remained undissolved in the fluid. Finally, therefore, this antialbumid was filtered off, washed by twice suspending it in water and filtering; and the combined filtrates were concentrated to three litres. At the end of this treatment the last trace of the biuret reaction had disappeared, — a point which was determined with the utmost care in all dilutions and with every possible modification of the test, and among others that of Neumeister relied upon by Loewi. The sulphuric acid was removed by means of hot saturated baryta water and the slight excess of barium by a few drops of dilute sulphuric acid. The fluid was concentrated to about two litres. Its content of nitrogen was then determined by the Kjeldahl method, and 100 c.c. pipetted off into each of eighteen small flasks. These were stoppered with cotton wool and placed in the steam sterilizer for an hour.

As it was essential that the animal's calorific needs should be fully supplied, and that the material used should be free from any albuminous substance, we employed for the purpose the best quality of lard and arrow-root starch. In each there was in the amounts fed daily a barely detectable trace of nitrogen. The food was prepared each

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TABLE SHOWING CONDITIONS AND RESULTS OF THE EXPERIMENT.

| DATE. 1902. | FOOD. | | | | URINE. | | FÆCES. N.[2] | Total output. N. | Balance of N. | Body weight. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N. | Starch. | Lard. | Calories.[1] | Vol. | N. | | av. per diem. | av. per diem. | | |
| April 23 | .. | .. | .. | .. | .. | .. | .. | | | .. | Fed bones. |
| " 24, 25 | .. | .. | .. | .. | .. | .. | .. | | | .. | Fasting. |
| " 26 | .. | .. | .. | .. | 70 | 1.93 | .. | | | 4.0 | " |
| " 27 | .. | .. | .. | .. | 40 | 1.84 | .. | | | 3.8 | " |
| " 28 | 4.5 | 30 | 20 | 300 | 120 | 5.46 | 0.80 | | | 3.8 | Vomiting. |
| " 29 | 4.5 | 35 | 35 | 450 | 80 | 4.54 | 0.80 | | | 4.0 | Fæces normal. |
| " 30 | 4.5 | 35 | 35 | 450 | 90 | 5.00 | 0.80 | 5.63 | −1.13 | 4.0 | " " |
| May 1 | 4.5 | 35 | 35 | 450 | 93 | 4.59 | 0.80 | | | 4.0 | " " |
| " 2 | 4.5 | 35 | 35 | 450 | .. | 4.54 | 0.80 | | | 4.0 | Vomiting; fæces normal. |
| " 3 | 4.5 | 35 | 35 | 450 | 140 | 3.95 | 0.80 | | | 3.8 | Vomiting; fæces soft. |
| " 4 | 4.5 | 35 | 35 | 450 | 71 | 4.45 | 0.80 | 5.22 | −0.72 | 3.8 | Fæces soft. |
| " 5 | 4.5 | 60 | 15 | 375 | 105 | 4.86 | 0.80 | | | 3.8 | Vomiting; diarrhœa. |
| " 6[3] | 4.5 | 60 | 15 | 375 | 83 | 3.63 | 0.80 | | | 3.6 | Diarrhœa. |
| " 7 | 4.5 | 60 | 20 | 420 | 110 | 4.04 | 0.80 | 4.58 | −0.08 | 3.8 | " |
| " 8 | 4.5 | 60 | 20 | 420 | 180 | 3.53 | 0.80 | | | 3.9 | Vomiting; diarrhœa. |
| " 9 | 4.5 | 60 | 20 | 420 | 160 | 3.90 | 0.80 | | | .. | Vomiting; diarrhœa. |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| " | 10 | .. | .. | .. | .. | .. | .. | .. | | | .. | Fasting. |
| " | 11 | .. | .. | .. | .. | 85 | 0.83 | .. | | | 3.5 | " |
| " | 12 | .. | .. | .. | .. | .. | .. | .. | | | .. | Fed bones. |

[1] The figures for the calorific value of the food are calculated for the starch and lard only, — that of the nitrogenous substances being unknown, — and are given merely to show that the animal's needs in this respect were fully supplied.

[2] The total nitrogen in the fæces between April 24 and May 13 (nineteen days) was 9.6 grams. For the sake of a safe basis of calculation it is assumed in the table that one twelfth of this amount, or 0.80 gram, was contained in the fæces of each day of feeding. The utilization of the nitrogen of the food was very poor, — only 83 per cent. The total weight of the air-dry fæces was 174 grams. The filter paper in the food amounted to 154 grams in all.

[3] On and after May 6 the food contained each day 2 grams of the following mixture: $Fe_2Cl_6$, 5 per cent; $K_2HPO_4$, 70 per cent; NaCl, 15 per cent; $CaHPO_4$, 6 per cent; $MgCl_2$, 4 per cent.

day by heating the contents of one of the flasks in a casserole and adding to it the starch which had previously been ground and mixed with a small quantity of cold water. While this mixture was still warm the lard was stirred in, together with enough finely divided filter paper to give a decidedly thick consistency to the mass. During the greater part of the period of feeding the animal ate this food in two portions daily, voluntarily, and even with apparent enjoyment, in spite of the intensely bitter taste of the proteid products. Toward the end of the experiment, however, considerable persuasion, and finally force, was necessary to induce complete ingestion.

For the investigation we employed a lean fox-terrier bitch. It was kept in a cage suitable to collecting any urine that might be passed. Catheterization was performed each morning. The fæces, consisting largely of filter paper, were united for analysis, bones being fed as a means of demarcation at the beginning and end of the nineteen days the animal was under observation. Whenever vomiting occurred the material was returned to the animal's stomach immediately, — which except for the filter paper would have been impossible. Although vomiting and diarrhœa occurred on the majority of the days of feeding, these conditions were not

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so marked as to invalidate the essential accuracy of the data presented.

Before and after the period of feeding the animal was allowed to fast for a few days in order that from the amount of nitrogen eliminated in the urine under these conditions the "hunger minimum" or waste of the tissue proteids might be determined.

The results of the investigation are given in the accompanying table (page 388).

The numerical results here presented are essentially similar to those obtained by Loewi. Even in the earlier days of feeding the nitrogen elimination was not increased by the full amount of the nitrogen ingested. From the ninth to the thirteenth day of feeding the animal was in nitrogenous equilibrium. Body-weight was maintained.

It seems fair to conclude, therefore, (1) that the nitrogenous substances in the diet were not immediately and wholly converted into urea and excreted; (2) that they were on the contrary to a considerable extent retained; and (3) that that portion which was expended (appearing in the urine) exerted a marked proteid-sparing action. These reactions seem to us, however, to afford a sufficient explanation of the facts without invoking the more radical hypothesis of proteid synthesis. The diminution in the nitrogen excretion from 1.9 grams in the fore period to 0.8 gram in the after period of fasting suggests that the protoplasmic waste had not been made good, and that the retention of nitrogen is not in itself a proof of proteid synthesis.

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# OBSERVATIONS ON THE URINE OF THE MUSKRAT (FIBER ZIBETHICUS).

By ROBERT BANKS GIBSON.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

OUR knowledge of the comparative chemistry of the urine is so fragmentary that it seems desirable to present a few observations which the writer has had the opportunity of making at Professor Mendel's suggestion on a rodent, Fiber zibethicus. Except in the case of a few of the domestic animals, only occasional statements regarding the composition of mammalian urine are to be found in physiological literature. It is assumed, from the data available, that in general the urine of carnivora corresponds in chemical makeup with that of man; whereas the urine of herbivora is characterized by an occasional deficiency in phosphoric acid, a reaction usually alkaline to litmus and a high content of hippuric acid and other aromatic compounds — all depending largely on the peculiar character of the diet of these animals.[1] That specific peculiarities in the metabolism of individual species may occur is illustrated in the case of the dog by the occurrence of kynurenic acid, a substance not yet found in any other animal.[2]

Regarding the dietetic habits of the muskrat there seems to be a difference of opinion among writers.[3] Our specimen, an apparently full-grown animal weighing 800 grams, readily ate such vegetable foods as apples, carrots, lettuce, and corn; and it likewise consumed animal tissues in the form of lean meat, pancreas, and liver.

**General characters of the urine.** — The *daily volume* of the urine varied from 54 to 205 c.c., the average of many days being 118 c.c. This quantity is considerably larger per unit of body weight than

[1] *Cf.* HOPKINS: SCHÄFER'S Text-book of physiology, 1898, i, p. 637–638.

[2] *Cf.* MENDEL and JACKSON: This journal, 1898, ii, p. 27.

[3] "It dives with great facility, feeding on the roots, leaves, and stems of water plants, and on the fruits and vegetables growing near the margins of the streams it inhabits" (Encyclopædia Britannica). BEDDARD writes: "Thus it appears that this rodent, like so many others, is largely carnivorous" (Cambridge Natural History, 1902, x, p. 478).



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the output of the common laboratory mammals. Thus Alezaïs[1] noted an average urinary secretion amounting to 8 per cent of the body weight in the guinea pig, a rodent somewhat comparable with the muskrat in regard to its dietetic habits and its structural characteristics. Whether this peculiarity is in any way related to the muskrat's aquatic habits could not be determined definitely. It was occasionally noted that after a bath the animal eliminated somewhat larger volumes of urine than at other times. But Sacc[2] has called attention to the relatively large volume of urine secreted by a terrestrial rodent, the marmot (Arctomys). He obtained as much as 25 to 36 per cent of the body-weight, and suggests that this noticeable renal activity is dependent on the insignificant loss of water by way of the lungs and skin in the rodents. Corresponding with the large output of urine in the muskrat, the *specific gravity* of the secretion was usually rather low, being in general below 1.010, and varying from 1.006 to 1.016. The urine emitted a faint musk-like *odor* which became more pronounced after heating. The *color* of the fresh urine was pale yellow, except during fasting or on a meat diet, when a deeper color was observed, even in dilute urine. When kept with thymol in a stoppered vessel exposed to the light, the urine gradually grew darker, becoming almost black. In the dark, however, no appreciable change in color was noticed. The *reaction* to litmus was acid during fasting and after carnivorous diet; at other times it was only faintly alkaline or neutral, always remaining acid to phenolphthalein. The average acidity to the latter indicator was equivalent to 70 c.c. $\frac{n}{10}$ acid per litre. Neumeister[3] has observed the urine of the primitive mammal, Echidna aculeata, to remain neutral after feeding meat and eggs, although it is usually assumed that the urine, even of herbivora, becomes acid in reaction to litmus on a carnivorous diet. The muskrat urine was ordinarily free from any sediment; in the concentrated alkaline secretion crystals of triple phosphate and of calcium oxalate were occasionally found. In the acid urines uric acid crystals separated out at times.

**Composition of the urine.** — Several litres of urine collected during a period of mixed diet yielded the analysis reported below.[4] Further

[1] ALEZAÏS: Archives de physiologie, 1897, ix, p. 576.

[2] SACC: Comptes rendus, 1872, lxxv, p. 1839.

[3] NEUMEISTER: Zeitschrift für Biologie, 1898, xxxvi, p. 77.

[4] The samples were preserved with thymol which Cronheim (Archiv für Physiologie, 1902, p. 262) has lately shown to be most satisfactory.

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data were obtained by collecting the urine in daily periods with various experimental diets.

The total N was estimated by the Kjeldahl-Gunning process; uric acid by the Ludwig-Salkowski method; urea by the method of Mörner and Sjöquist, which seemed trustworthy in the absence of any considerable quantities of hippuric acid;[1] ammonia by Folin's method; oxalic acid by the process of Autenrieth and Barth;[2] and the other constituents by the commonly described methods.

ANALYSIS OF MUSKRAT URINE.

| | Grams per litre. | | Grams per litre. |
|---|---|---|---|
| Total N | 4.62 | Phosphoric acid ($P_2O_5$) | 0.69 |
| Urea | 9.04 | $P_2O_5$ with alkali earths | 0.45 |
| Hippuric acid | 0.25 | Sulphuric acid ($SO_3$) | 0.48 |
| Uric acid | 0.22 | Ethereal $SO_3$ | 0.02 |
| Oxalic acid | 0.04 | Chlorine (as NaCl) | 0.40 |

To ascertain something regarding the average daily output of the more important constituents, the urine was carefully collected for four days during a mixed diet somewhat larger in quantity and richer in meat than in the case above. The output for this period was 423 c.c., having a specific gravity of 1.011. The data obtained follow.

| | Grams per day. | Grams per litre. |
|---|---|---|
| Total nitrogen | 0.808 | 7.62 |
| Urea | 1.635 | 15.43 |
| Uric acid | 0.037 | 0.35 |
| Ammonia | 0.008 | 0.08 |
| Xanthin bodies | .... | 0.01 |
| Phosphoric acid ($P_2O_5$) | 0.088 | 0.83 |

[1] *Cf.* MÖRNER: Skandinavisches Archiv für Physiologie, 1903, xiv, p. 2[illegible].

[2] AUTENRIETH and BARTH: Zeitschrift für physiologische Chemie, 1902, xxxv, p. 327.

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The distribution of nitrogen in the various types of nitrogenous constituents in the two experiments was as follows:—

| | Exp. 1. | Exp. 2. |
|---|---|---|
| | per cent. | per cent. |
| Urea nitrogen . . . . . . . | 91.3 | 94.3 |
| Uric acid nitrogen . . . . . | 1.5 | 1.5 |
| Ammonia nitrogen . . . . . | .... | 0.9 |
| Hippuric acid nitrogen . . . | 0.4 | .... |
| Xanthin bodies nitrogen . . . | .... | 0.2 |
| Undetermined . . . . . . . | 6.8 | 3.1 |
| | 100.0 | 100.0 |

**Comments on the analytical data.** — From the figures presented the nitrogenous metabolism of the muskrat is seen to resemble quantitatively that of the guinea pig, the average output per hundred grams of animal being 0.1 gm. N.[1] The small proportion of nitrogen in the form of ammonia compounds (one per cent) corresponds with previous experience on other herbivora[2] and with prevailing ideas regarding the origin of ammonia in metabolism. Although uric acid was excreted in noticeable quantity, the figures are no larger than those obtained by Mittelbach for the urine of various herbivora.[3] He found quantities ranging from 90 to 450 mgm. per litre. It will be noted above that an increase of uric acid in the muskrat urine followed the more liberal introduction of meat into the diet. Neumeister[4] failed to find uric acid in the urine of Echidna under similar conditions. The elimination of phosphoric acid noted is of interest in view of the current statements regarding the low output in the herbivora.[5]

Since in the dog and cat the ingestion of tissues rich in nucleic acid is followed by a large elimination of allantoïn, pancreas feeding was tried in the case of the muskrat, with negative results. Similar

[1] ALEZAÏS: *Loc. cit.*, p. 579.

[2] HUPPERT: Analyse des Harns, 1898, p. 42.

[3] MITTELBACH: Zeitschrift für physiologische Chemie, 1888, xii, p. 466.

[4] NEUMEISTER: *Loc. cit.*

[5] *Cf.* SACC: *Loc. cit.* (marmot). Also BERTRAM: Zeitschrift für Biologie, 1878, xiv, p. 335.

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experience has followed earlier experiments on rabbits in this laboratory.[1] Kynurenic acid was also missing and creatinine could not be isolated. The urine contained urobilin ; at times bile pigments were present, giving Huppert's test and imparting a greenish tint to the fluid. Proteids and sugar were never detected.

[1] MENDEL, UNDERHILL, and WHITE: This journal, 1903, viii, p. 399.

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# SALIVARY DIGESTION IN THE STOMACH.[1]

By W. B. CANNON and H. F. DAY.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN stating the functions of saliva the common reference is to its importance as a lubricant in facilitating the movement of the parts of the mouth upon one another and in aiding the passage of the food through the œsophagus. There is a widespread impression that the chemical function is slight. Saliva can indeed change starch to sugar, but during mastication there is little time for amylolysis, and in the stomach the action of ptyalin is soon stopped by the acid gastric juice. Such is the view commonly expressed.[2] This paper is a report of a critical inquiry into the reasons for this view, and an experimental study of the degree of salivary digestion in the cardiac and pyloric ends of the stomach.

To support the conclusion that salivary digestion in the stomach is slight, certain studies of gastric contents after feeding starches are cited, and attention is called to the common conceptions of the effect of gastric peristalsis. Brücke[3] found in the stomach contents of dogs killed one to five hours after eating starch paste or mush, no sugar or only slight amounts, unless it was already present in the food ingested. In 1877 v. Mering[4] concluded, after feeding starch paste to dogs, that the saliva of dogs was without digestive influence, evidently because ptyalin was absent, and that in man, although ptyalin must be considered, it is of secondary importance. Observations on human beings by Ewald and Boas[5] in 1886 seemed to

[1] A preliminary report of this research was presented to the American Physiological Society in December, 1902, and was printed in the proceedings of the meeting, This journal, 1903, viii, p. xxviii.

[2] See, for example, NEUMEISTER, Lehrbuch der physiologischen Chemie, Jena, 1897, pp. 287, 288.

[3] BRÜCKE: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, Wien, 1872, lxv, p. 144.

[4] v. MERING: Archiv für Physiologie, 1877, p. 393.

[5] EWALD and BOAS: Archiv für pathologische Anatomie und Physiologie, und für klinische Medicin, 1886, civ, p. 296.



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sustain v. Mering's contention, for after giving patients starch paste to drink only slight amounts of sugar were found in the stomach. The next year Seegen[1] reported experiments in which he fed dogs carbohydrate food of diverse forms (flour cakes, potatoes, rice), and yet in the gastric contents failed to find more than a trace of sugar.

These chemical experiments tending to prove that saliva has only slight action in the stomach were supported by observations and theories of the churning effect of gastric peristalsis. Beaumont's[2] well-known description of the movement of the bolus along the gastric walls, and Brinton's[3] theory of peripheral and axial streaming of the food in the stomach offered reasons for believing that all the food is rapidly mixed with gastric juice and is thus in a short time made acid throughout. The duration of salivary digestion must therefore necessarily be brief.

The conclusion from the above evidence that salivary digestion in the stomach is slight is not above criticism. In the first place the experiments of Brücke, v. Mering, and Seegen were performed upon dogs. The absence of sugar from the stomach, noted by these observers after giving a carbohydrate meal, is amply explained, as v. Mering admitted, by the fact that the dog's saliva does not possess a diastatic ferment.[4] Even with ptyalin present in the saliva, which was the case in the experiments of Ewald and Boas on human beings, the starch paste as a test food would be objectionable. It is not only not palatable, but is in a state to be swallowed immediately without being chewed. There are thus two reasons for its not being mixed with sufficient saliva to cause noteworthy amylolysis. Inasmuch as there was no ptyalin, or almost no ptyalin, mixed with the food given in all these experiments, it is clear that they do not furnish direct evidence that salivary digestion in the stomach must be slight.

The indirect evidence against gastric amylolysis derived from Beaumont's observations and Brinton's reasoning as to mixing currents, is also subject to criticism. In 1898, Cannon[5] brought forward proof that neither Beaumont's nor Brinton's account of the movement of the food in the stomach was correct. A marked difference was observed between the effects of the mechanical activities of the pyloric

[1] SEEGEN: Archiv für die gesammte Physiologie, 1887, xl, p. 46.
[2] BEAUMONT: Physiology of digestion, Plattsburgh, 1833, p. 110.
[3] BRINTON: The diseases of the stomach, Philadelphia, 1865, p. 24.
[4] See GRÜTZNER: Archiv für die gesammte Physiologie, 1876, xii, p. 285.
[5] CANNON: This journal, 1898, i, p. 378.

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and the cardiac portions of the stomach. In the cardiac portion there is no peristalsis, and the food, held in the tonic grasp of the gastric musculature, shows no signs of movement; in the pyloric portion, on the other hand, gastric peristalsis mixes the food thoroughly with the digestive juices. Little pellets of starch paste lying in the cardiac end immediately after the food is ingested may be seen with the X-rays in the same relative positions for almost two hours; the pellets in the pyloric end are moved to and fro by the passing waves of constriction. There is moreover a marked difference in the appearance of the contents of the two parts of the stomach after digestion has proceeded for even thirty minutes: the food in the pyloric end is usually far advanced toward chymification; the food in the cardiac end has still its original appearance, which it may retain even an hour and a half after ingestion. This observation that the food in the cardiac end of the stomach is not moved, and therefore is not mixed with the gastric juice, suggested that the cardiac contents might retain their original chemical reaction for a considerable period. Tests made on several cats and dogs, from one to one and a half hours after feeding, showed that the contents of the pyloric end were invariably strongly acid; the surface of the cardiac contents was also acid, but the internal mass in the cardiac end remained unchanged in its reaction. Inasmuch as salivary digestion may continue so long as free acid is absent, the conclusion was drawn that salivary digestion might proceed in the fundus for an hour and a half or longer without interference by the acid gastric juice.

The observations and the conclusion just detailed have been confirmed by Oehl,[1] and still more completely by Heyde working under Grützner[2] in Tübingen. Rats, rabbits, guinea pigs, and cats were fed by Heyde with different kinds of food in definite amounts and killed at different intervals after eating. The stomachs were carefully removed and frozen; sections were made through the frozen gastric contents, and acid indicators mixed with the food revealed at once the extent of acidification. The inner layers of the food in the cardiac end retained for hours a neutral or weakly alkaline reaction. Only the outer layers were slightly acidified and digested. Thus the diastatic ferment of the saliva may continue its action in the fundus for a long period wholly unhindered.

The difference in motor activities between the cardiac and pyloric

[1] Oehl: Archives italiennes de biologie, 1899, xxxii, p. 114.

[2] Grützner: Reprint from Deutsche Medizinal-Zeitung, 1902, No. 28.

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ends of the stomach has been noted also in human beings. Beaumont's observations on Alexis St. Martin hint at a difference, and v. Pfungen[1] and Moritz[2] have made it certain that in man the peristalsis is confined to the pyloric end. Since Beaumont's time few observations have been made on the movements of the food in the human stomach. In 1897, however, Hemmeter[3] stated that after many experiments on animals and investigations through fistulæ into the human stomach, he found no confirmation of Beaumont's and Brinton's views as to mixing currents. In 1900 Hemmeter[4] repeated this statement and affirmed that Cannon's assertion that the food is churned by peristaltic waves only in the pyloric portion was correct. After thus bringing confirmatory data to prove the fundus free from peristalsis and after entirely rejecting the idea of mixing currents, he still declares that the contents of the fundus are soon mixed with the gastric secretions. The only explanation offered for this conclusion is that food taken from the stomach by means of an Einhorn bucket always contains gastric juice. Since the dorsal wall of the stomach in man slopes forward from the cardiac opening, a tube introduced through the œsophagus comes immediately in contact with the dorsal wall, and will remove first the food lying in proximity to the secreting mucous membrane. Clearly this faulty evidence allows no conclusion to be drawn as to the presence of acid in the interior of the cardiac mass, and such evidence cannot be accepted as contradicting the accumulated testimony, from much more exact methods, that the internal food in the fundus long remains unmixed with acid.

The discussion thus far has led to two conclusions: (1) that the reasons commonly given to prove salivary digestion possible in the stomach for only a short time are by no means convincing, and (2) that in the fundus, a reservoir in which the food may rest for hours unmoved, salivary digestion may indeed continue for a long period without interruption.

Several researches not widely quoted lend support to the view that salivary digestion in the stomach may be of importance. As long ago as 1880, von den Velden[5] pointed out that free hydrochloric acid did not appear for almost an hour after eating breakfast and for almost two

[1] v. Pfungen: Centralblatt für Physiologie, 1887, i, p. 220.

[2] Moritz: Zeitschrift für Biologie, 1895, xxxii, p. 339.

[3] Hemmeter: Diseases of the stomach, Philadelphia, 1897, p. 86.

[4] Hemmeter: *Loc. cit.*, second edition, 1900, pp. 85 and 88.

[5] v. d. Velden: Deutsches Archiv für klinische Medicin, 1880, xxv, pp. 105 and 111.

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hours after eating a full mid-day meal. Austen[1] in 1899, after studying one of his students, stated that for a period of at least one hour after an ordinary meal the albuminous matter of the food united with the hydrochloric acid of the gastric juice as fast as the acid was secreted and thus prevented it from stopping the digestion of starch. Hensay[2] and Müller[3] were the first to present quantitative measurements of the amounts of sugar and dextrins which might be formed in the stomach when food is carefully chewed. These observers fed rice mush made pleasing to the taste with meat extract and butter. After a certain time, usually one half hour, they pressed out the gastric contents as completely as possible and secured the remnant by washing. They found that a large part of the expressed carbohydrates, 59.4 to 79.6 per cent, had been made soluble by the saliva. Of the dissolved carbohydrates over one half, even two thirds, consisted of maltose and of dextrins closely related to maltose, and the remainder of dextrins more nearly related to starch.

The four reports just mentioned combine to show that the common teaching that saliva must be unimportant in the stomach, because its action is quickly stopped, is not correct. Saliva certainly may be important in the digestion of starch if the food is well chewed and thus thoroughly mixed with the ferment. The observers who have brought forward these positive results have not, it will be noted, regarded the differences between the pyloric part of the stomach and the fundus. It was the purpose of the research here reported to study the conditions of salivary digestion particularly in the two portions of the stomach: in the active pyloric portion where the food is soon mixed with the acid juice; and in the quiet cardiac reservoir where the food lies unmoved for a long time until it begins to be forced gradually into the churning mechanism at the pyloric end.

## Method.

The cat was the animal used in this investigation. The stomach of the cat, as observations with the X-rays have proved,[4] is like the

[1] Austen: Boston medical and surgical journal, 1899, cxl, p. 325.

[2] Hensay: Münchener medicinische Wochenschrift, 1901, xlviii p. 1208.

[3] Müller: Sitzungsberichte der physikalisch-medicinische Gesellschaft, Würzburg, 1901, p. 4.

[4] Cannon: This journal, 1903, viii, p. xxii; Roux and Balthazard: Archives de physiologie, 1898, xxx, p. 85; Williams: The Röntgen ray in medicine and surgery, New York, 1901, pp. 360–372.

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stomach of the dog, rat, rabbit, guinea pig, and man, in being separable into two parts, — the quiet cardiac end and the active pyloric end. Moreover the mucosa of the cat's stomach resembles that of the dog and of man, not only in structure but also in pouring out an active secretion from almost every part of its surface.[1] The cat may be regarded, therefore, as a fairly typical animal for the purposes of the present investigation.

From twenty-four to thirty-six hours previous to an experiment the cat was allowed to fast, in order that the stomach might be free from food and prepared to receive the test meal. Crackers, examined and found free from sugar, were used as the test food. Unless otherwise stated a uniform amount of coarsely powdered crackers, 30 gms., was mixed with a uniform amount of filtered human saliva, 100 c.c. The mixture has a consistency of thick mush, similar to that of food chewed to a degree suitable for swallowing. The food thus prepared was given in one of two ways: it was either mixed in small amounts and fed immediately to the animal, or the total amount of crackers was mixed with the total amount of saliva and introduced at once into the stomach by a stomach tube. As the results were the same with both methods, the latter, because more expeditious, was usually employed.

After the food was in the stomach the animals were allowed to live one half hour, one hour, one and a half hours, or two hours. At the end of the time the animal was quickly etherized, the abdomen opened along the middle line and along a line toward the left parallel with the greater curvature of the stomach. With a curved hook a ligature was passed around the stomach at the region where the peristaltic waves start toward the pylorus,[2] and tied firmly. Thus the contents of the cardiac and pyloric portions were separated. The pylorus and cardia were next ligatured and the stomach removed with as little handling as possible.

Openings were now cut in the stomach wall and the contents of each part were emptied into an evaporating dish. The food in the pyloric end invariably had a consistency of thin mush. Occasionally hair or remnants of meat were found in the pyloric end; cases in which these disturbing factors were present have been excluded from this report.

[1] OPPEL: Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere, erster Theil, Der Magen, Jena, 1896, pp. 408, 443, 463; CARVALLO: Article "Estomac," Dictionnaire de physiologie, edited by Richet, Paris, 1902, v, p. 818.

[2] CANNON: This journal, 1898, i, p. 364.

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The consistency of the food in the cardiac end was very different from that of the food in the pyloric end. It was not fluid, like the food in the pyloric end, and it often retained its shape sufficiently to permit the surface food to be separated from the internal mass.

The contents in the evaporating dishes, if not fluid, were slightly diluted with water, and the enzyme action quickly stopped by heating to the boiling point. The food was now evaporated to dryness by steam heat; the dry residue was ground to a fine powder in a mortar and kept in a desiccator until its weight was constant. One gram, removed from each part of the dried and powdered stomach contents, was mixed with 100 c.c. distilled water. After standing for about one half hour the mixture was filtered, and the filtrate was poured several times through the residue on the filter. In each instance 25 c.c. of the filtrate were taken for the sugar test. The sugar content, determined according to Allihn's method,[1] was estimated as maltose.[2] It was thus possible to know the percentage of sugar in the dry residue of the stomach contents.

## Factors to be Considered.

The first and most important of the factors to be considered is the presence of free hydrochloric acid. As already stated, experiments in 1898 proved that free acid was absent from the middle of the cardiac mass for one and a half or two hours after eating. Similar tests made during the present research have confirmed the earlier observations. At the end of one half hour after a carbohydrate meal, free hydrochloric acid is present in the pyloric end of the stomach, but at the end of two hours there is no free acid present in the middle of the cardiac food.

Another factor to be regarded is the rapidity of salivary digestion. The common statements as to the speed of the change from starch into sugar are based upon observations on starch paste.[3] Tests with other forms of starchy food show in some cases a much slower rate.[4] Observations made on the test material used in this research, under-

[1] Allihn: Zeitschrift für analytische Chemie, 1883, xxii, p. 448.

[2] Musculus and v. Mering: Zeitschrift für physiologische Chemie, 1878–9, ii, p. 409; Ewald and Boas: *Loc. cit.*, p. 297.

[3] Chittenden and Ely: Journal of physiology, 1882, iii, p. 327.

[4] Hammarsten: Jahresberichte über die Fortschritte der Thierchemie, 1871, i, p. 187.

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going salivary digestion *in vitro* at 38° C., show that in seven minutes four fifths of the amount of sugar found at the end of an hour is already present. To be sure, under these conditions the accumulation of the products of the digestion inhibits the rapidity of the action of the ferment as time passes,[1] but it is clear that salivary digestion is sufficiently rapid to cause a considerable amylolysis before the acid is secreted even in the pyloric end to a degree preventing further activity of the ptyalin.

Control experiments were made to discover if in the stomach or in the treatment of its contents there was any factor which, aside from the saliva, would result in any considerable change from starch to sugar. Crackers mixed with distilled water were fed to animals, and after an hour the stomach contents were treated in the usual routine. Under these conditions only the slightest traces of a reducing agent were discovered.

## Results.

The difference in salivary digestion in the two ends of the stomach after different periods of time is shown in the table on page 404.

Several matters are to be noted in reference to the figures in this table. There is a remarkable diversity in the amount of maltose present in different cases having the same period of digestion, and there is no very uniform increase in the figures as the period of digestion is prolonged. Probably numerous agents are concerned in producing this diversity, but control experiments carried on *in vitro* indicate that the most important cause is the variation in the activity of the saliva at different times, — a fact to which attention has been called by several observers.[2] It is evident that the figures in these cases cannot be compared absolutely; in each instance, however, the ratio of the change in the two parts of the stomach may be taken and these ratios will serve for comparisons.

The ratios derived from the figures show that there is in every case a greater percentage of sugar present in the cardiac end than in the pyloric end. Two considerations serve to prove that the actual differences between the *total amounts* of sugar produced in the two ends of the stomach are notably greater than the differences between

[1] Lea: Journal of physiology, 1890, xi, p. 239.

[2] See Hofbauer: Archiv für die gesammte Physiologie, 1897, lxv, p. 503; Chittenden and Richards: This journal, 1898, i, p. 461; Oehl: Memorie del reale istituto lombardo di scienze e lettere, 1902, xix, p. 135.

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the sugar present in unit volumes of the dried contents. In the first place, as previously stated, the pyloric contents are invariably more fluid than the cardiac contents: it follows, therefore, that, since a

In this and in subsequent tables each number represents the fractional gram of maltose present in one gram of dried contents of different parts of the stomach, after different periods of time.

| Period. | Date. | Pyloric end. | Cardiac end. | Ratio. |
|---|---|---|---|---|
| ½ hr. | July 15 | 0.352 | 0.363 | 10 : 10.3 |
| | Aug. 1 | 0.171 | 0.209 | 10 : 12.2 |
| | Jan. 14 | 0.338 | 0.401 | 10 : 11.9 |
| | | | Average ratio . . . . . . . | 10 : 11.5 |
| 1 hr. | July 18 | 0.293 | 0.472 | 10 : 16.1 |
| | Aug. 1 | 0.095 | 0.174 | 10 : 18.3 |
| | Jan. 30 | 0.111 | 0.206 | 10 : 18.5 |
| | | | Average ratio . . . . . . . | 10 : 17.6 |
| 1½ hrs. | July 11 | 0.360 | 0.506 | 10 : 14.0 |
| | " 18 | 0.244 | 0.384 | 10 : 15.7 |
| | " 26 | 0.262 | 0.295 | 10 : 11.2 |
| | Aug. 1 | 0.135 | 0.244 | 10 : 18.0 |
| | Jan. 14 | 0.291 | 0.360 | 10 : 12.3 |
| | | | Average ratio . . . . . . . | 10 : 14.2 |
| 2 hrs. | July 15 | 0.315 | 0.455 | 10 : 14.4 |
| | " 26 | 0.291 | 0.322 | 10 : 11.0 |
| | | | Average ratio . . . . . . . | 10 : 12.7 |

larger amount of fluid must be evaporated from the pyloric food than from the cardiac food, in order to secure the dried masses, there is relatively less sugar in a unit volume of the original pyloric food in

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comparison with a unit volume of the original cardiac contents than the ratios given in the tables would indicate. For this reason, therefore, the actual disparity of sugar production in the two ends of the stomach is greater than that seen in the dried contents. The second factor increasing these differences is the inequality in the cubic contents of the two ends of the stomach. After a full meal the cardiac end holds by far the greater part of the food. Several observations on the relative capacity of pyloric and cardiac portions of the stomach, as shown by their contents after the ingestion of about 100 c.c. of food, brought forth the average ratio of 1 to 5. In order to get the ratio of the total amounts of sugar in the two regions it is necessary to multiply the cardiac figure by 5; from this factor alone, therefore, the ratios given in the above table change so that they are as 10 to 57.5 for one half hour, as 10 to 88 for one hour, as 10 to 71 for one and one half hours, and as 10 to 63.5 for two hours.

It is to be observed that after one half hour the amount of sugar in equal parts of the dried contents of the two regions of the stomach is almost the same, that the greatest difference between the two regions appears in the estimates for one hour, and that the ratios for an hour and a half and for two hours again approach unity. Without much doubt the reason for the percentage sugar content being almost the same in the two portions of the stomach at the end of a half hour is that within that time the sugar formation takes place at almost equal rates in the two portions, *i. e.*, for some time after the ingestion of food the ptyalin is not inhibited even in the pyloric region by the appearance of free acid. Every examination of cases in which digestion had proceeded for a half hour demonstrated that free acid was already present in the pyloric contents. Salivary digestion in the pyloric end of the stomach was therefore at a standstill. That salivary digestion does not cease, however, in the pyloric end until toward the end of the half hour is shown by the large amount of sugar produced compared with that in the cardiac end. On the other hand, the fact that there is a difference between the sugar percentages in the two portions shows that the free acid in the pyloric end has had an inhibitory effect. This inhibitory effect is more marked if the speed of action of the ptyalin is retarded (as, for example, by diluting it), for then the acid has opportunity to check the activity before considerable change has occurred. Thus in the stomach of an animal fed 30 gms. crackers mixed with 100 c.c. diluted saliva (saliva 25 c.c., water 75 c.c.), the ratio of sugar percentages at the end of a half hour was

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not near 10: 11.5, the average when undiluted saliva was given, but was as 10 to 20, a ratio approximating that found at the end of an hour when undiluted saliva is used. Under ordinary circumstances in human beings the saliva is thus diluted by drinking at meals. If the results secured with the cat may be transferred to the human subject (a matter to be considered later), the factor of dilution would certainly enhance the relative value of the fundus as a region for salivary digestion.

The change in the ratios in the above tables, from 10 : 17.6 at the end of an hour, to 10 : 12.7 at the end of two hours, can be considered only after evidence of other changes has been presented. The evidence for these changes will now be given.

It is certain that the sugar formed is in solution, and it is highly probable that this solution may diffuse from a region in which it is more concentrated into a region in which it is less concentrated. It may also pass to the lowest position which a fluid may take in the stomach. That such changes in the sugar concentrations in the stomach occur is evidenced by the following observations. In four animals digestion was allowed to proceed for one and a half hours. The contents of the cardiac end were then removed in two parts: an external mass from near the ventral wall of the stomach, which was lowest during digestion ; and the internal mass, dorsal in its relation to the first mass. When these masses were dried the sugar percentages were as follows:

| Date. | External food (from lowest part). | Internal food (from higher part). |
|---|---|---|
| July 11 . . . . | 0.511 | 0.500 |
| " 18 . . . . | 0.410 | 0.357 |
| " 26 . . . . | 0.326 | 0.263 |
| Jan. 14 . . . . | 0.360 | 0.281 |

These results indicate that as the sugar solution is formed it passes into a dependent portion of the stomach. Further data on this point were secured by having an animal rest on the left side during a digestive period of an hour. Since the cat's stomach lies more nearly transverse than longitudinal in the body, the position on the left side caused the cardiac end to lie below the pyloric. The sugar

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percentages of the food in the most dependent portion and in the interior are as follows:

| Date. | External food (from lowest part). | Internal food (from higher part). |
| --- | --- | --- |
| Aug. 4 . . . . | 0.398 | 0.301 |
| Dec. 15 . . . . | 0.482 | 0.468 |
| Jan. 15 . . . . | 0.481 | 0.422 |

When an animal lies on its *right* side during an hour of digestion, opposite results are secured from the cardiac end, thus:

| Date. | External food (from highest part). | Internal food (from lower part). |
| --- | --- | --- |
| Jan. 14 . . . . | 0.291 | 0.423 |

As already noted, at the end of an hour or an hour and a half the food near the wall in the cardiac end of the stomach almost invariably shows the presence of free acid, while the internal food of this region retains its original reaction. Evidently in the cases cited the internal region, favorable for amylolysis, contains less sugar than the dependent region, in which the process is hindered or prevented. The most reasonable explanation for a larger amount of sugar near the dependent gastric wall than in the interior of the food mass, is that the sugar solution runs into the lowest region from the effect of gravity.

Inasmuch as the sugar solution passes thus from the midst of the food in the stomach to the gastric wall, it is probable that diffusion also occurs from the cardiac region into the pyloric region, in which sugar is present in less amount. The fact of diffusion can readily be demonstrated by feeding first meat, and later starchy food mixed with saliva. Under such circumstances the meat is found crowded against the greater curvature, the starchy food lies along the lesser curvature, and between the two foods a perfectly clear separation is possible. If the meat by itself causes no reduction of copper, whatever reduction it may cause subsequent to its being in the stomach alongside of food undergoing amylolysis must be due to diffusion of sugar

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into the meat. An animal was fed thus with shredded canned salmon and later with crackers mixed with saliva; and the meat taken at the end of an hour from near the surface in the cardiac region, fully a centimetre and a half from the starchy food, although originally giving no reaction for sugar, contained about 0.7 per cent sugar calculated as maltose. Since the sugar in the starchy food was only 27 per cent, the relative per cent of sugar in the meat was almost 3 per cent. Diffusion of sugar into the meat had evidently taken place to a considerable degree.

A further result of the solution of the sugar is the possibility, in this state, of its being absorbed. Brandl[1] and v. Mering[2] have proved that sugars may be absorbed from the stomach, and have shown that within limits the rate of absorption increases with the increase of concentration of the solution. Inasmuch as the sugar is present in the cases under consideration in somewhat high concentrations, it is certainly very likely that some sugar is absorbed directly from the stomach.

Only these facts of diffusion and absorption will explain certain observations repeatedly made in the course of the experiments, namely, that control digestions *in vitro* almost invariably resulted in the production of more sugar than could be found in the cardiac end of the stomach. Typical examples of the difference are as follows:

| Date. | Control food (*in vitro*). | Cardiac food. |
|---|---|---|
| July 18 | 0.473 | 0.384 |
| " 26 | 0.309 | 0.295 |
| " 11 | 0.436 | 0.506 |
| Aug. 1 | 0.309 | 0.244 |

In these instances there is no free acid present in the cardiac end to prevent the action of the ptyalin; and, moreover, the action of the ptyalin is not retarded by its products, for there is not so great an accumulation of sugar in the fundus as in the control dishes. The

[1] Brandl: Zeitschrift für Biologie, 1892, xxix, p. 277.

[2] v. Mering: Verhandlungen des xii Congresses für innere Medicin zu Wiesbaden, 1893, p. 471.

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facts already presented justify the conclusion that there is a continuous formation of sugar in the fundus, that as the sugar is formed it diffuses from the region of greater concentration into regions of less concentration, and that it is absorbed as it comes in contact with the gastric walls. Thus the differences between sugar percentages in the two ends of the stomach would become less as time elapsed, and thus there might readily be less sugar present in the cardiac end of the stomach than in an artificial digestion from which the products do not pass away.

It is probable that as the sugar diffuses from the region of greater concentration into regions of less concentration a certain amount of the ptyalin is carried away with it. That this loss is not serious is shown by making a watery extract of the cardiac contents and testing its amylolytic power. Such extracts were made of pyloric contents, and of food near the surface and in the interior of the cardiac end after digestion had proceeded for an hour. Tested with starch paste, blue with iodine, these extracts gave the following results: pyloric food, no change of color in four hours; surface of cardiac food, slight change of color; interior of cardiac food, complete disappearance of color in a short time. Manifestly ptyalin was still active in the cardiac end of the stomach.

Further evidence of absorption was secured by feeding two animals with equal amounts of the same food mixed with equal amounts of the same saliva, and examining the stomach contents at different intervals after the food was given. The experiment was tried twice with the following results:

| Date. | Period. | Cardiac food. | Pyloric food. |
|---|---|---|---|
| July 15 . . . | ½ hours | 0.363 | 0.352 |
| | 2 " | 0.455 | 0.315 |
| Aug. 1 . . . | ½ " | 0.209 | 0.171 |
| | 1½ " | 0.244 | 0.135 |

In these two cases the cardiac sugar content increases as time goes on; naturally it would be expected that as the cardiac food passes into the pyloric end the pyloric sugar content also would increase; instead, however, as time goes on there is a decrease in the percentage in the pyloric end. These two cases are not sufficient to allow sure

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conclusions to be drawn, but they indicate that sugar passes out of the pyloric end of the stomach more rapidly than the undissolved portions of the food. A selective action at the pylorus is hardly to be expected, for the food that comes to it is not a mixture of hard particles in fluid, but a uniform creamy substance. Active absorption at the pyloric end, for which the peristalsis is especially favorable, is suggested as an explanation of the results which these two cases furnish.

**Effect of position on sugar formation.** — It is important to know if position has an effect on the mixing of the gastric contents with the gastric secretions, and if thereby salivary digestion is modified. Observations were made on animals caused to rest on the right side or on the left side during the period of digestion. As the full stomach in the cat lies nearly transverse, the long axis of the organ was nearly vertical in either case. The results secured were as follows:

| Animals on Left Side. | | | |
|---|---|---|---|
| Date. | Pyloric end. | Cardiac end. | Ratio. |
| Aug. 4 . . . | 0.191 | 0.350 | 10 : 18.3 |
| Dec. 15 . . . | 0.435 | 0.475 | 10 : 10.9 |
| Jan. 15 . . . | 0.227 | 0.451 | 10 : 19.8 |
| | | Average ratio . . . . | 10 : 16.3 |
| **Animals on Right Side.** | | | |
| Dec. 15 . . . | 0.306 | 0.473 | 10 : 15.4 |
| Jan. 14 . . . | 0.251 | 0.357 | 10 : 14.2 |
| | | Average ratio . . . . | 10 : 14.8 |

Examination of these results reveals no very marked differences in the ratios of sugar content when the long axis of the stomach is reversed in direction. If the animal is on the left side, with the cardiac end lower than the pyloric end, there is, however, more sugar present in the food in the fundus than is the case when the opposite relation of the parts is maintained.

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**Effect of variations in the food, and massage.** — When the food is liquid, it is to be expected that the gastric juice will be more readily and more uniformly mixed with the food than when the food is present in somewhat viscid masses. Crackers (30 gms.) were ground to a coarse powder and mixed with 150 c.c. filtered saliva. The mixture was about as fluid as the chyme ordinarily seen in the pyloric end of the stomach. After one hour the percentage sugar content in dried food from the two ends of the stomach was as follows:

| Date. | Pyloric end. | Cardiac end. | Ratio. |
|---|---|---|---|
| Dec. 15 . . . | 0.427 | 0.519 | 10 : 12 |

The average ratio for one hour with the usual test food was 10 : 17.6; with the liquid diet there is a considerable decrease in the disparity.

Similarly small amounts of food in the stomach would naturally be more quickly and uniformly permeated by the gastric secretions, and the differences to be observed when large amounts are given would not under these circumstances be expected. Animals were fed one half the usual test meal, — 15 gms. crackers, 50 c.c. saliva, — and digestion allowed to proceed for one hour. The following results show the sugar production in the two parts of the stomach:

| Date. | Pyloric end. | Cardiac end. | Ratio. |
|---|---|---|---|
| Aug. 4 . . . | 0.369 | 0.349 | 10 : 9.4 |
| Jan. 30 . . . | 0.296 | 0.284 | 10 : 9.6 |
| | | Average ratio . . . . | 10 : 9.5 |

In these cases more sugar is present in a unit weight of dried pyloric content than in a unit weight of dried cardiac content, a fact difficult of explanation.

A difference in the ratios similar to that observed when the amount of food was small was seen in two instances in which the upper abdomen was massaged at intervals of about five minutes during one

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hour of digestion. The sugar content in these instances was as follows:

| Date. | Pyloric end. | Cardiac end. | Ratio. |
|---|---|---|---|
| Aug. 4 . . . | 0.484 | 0.391 | 10 : 8.1 |
| Dec. 15 . . . | 0.493 | 0.423 | 10 : 8.6 |
| | | Average ratio . . . | 10 : 8.4 |

In these instances, as when liquid food or a small amount of food was given, the sugar content of the two ends of the stomach was more nearly the same than in the usual cases with large amounts of semi-solid food resting in the stomach undisturbed. The larger percentage of sugar in the pyloric end, when little food was given and when the stomach after a full meal was massaged, was not expected and cannot at present be explained.

**Effect of combining proteid with carbohydrate food.** — From the work of Pawlow and his school[1] it is to be expected that after the introduction of flesh food, the character of the secretion of the gastric juice will change; there will be in the early stages of digestion a more abundant flow of gastric juice, with a concomitant greater production of hydrochloric acid, than is normal when carbohydrates alone are ingested. On the other hand, as pointed out by Chittenden and Smith,[2] proteid not only favors the diastatic action of ptyalin by combining with hydrochloric acid so as to delay the appearance of the free acid, but also seems to act, in the form of a small percentage of acid proteid, as a direct stimulant to diastatic action. The effects of proteid favorable to diastatic activity might therefore counterbalance the extra production of acid destructive to that activity. Such in fact was the result of experiment. A cat was fed 45 gms. of thoroughly mixed fish and crackers (25 gms. salmon, 20 gms. crackers) and 100 c.c. diluted saliva (50 c.c. saliva, 50 c.c. water). After one hour the stomach was examined in the usual manner. No free acid was found in the cardiac end, and in the pyloric end there was only a slight

[1] Pawlow: The work of the digestive glands, London, 1902, pp. 31, 34, and 35.

[2] Chittenden and Smith: Studies from the laboratory of physiological chemistry, Sheffield Scientific School of Yale College, 1885, i, p. 33.

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discoloration of the test paper. The food, treated in the manner already described, yielded the following amounts of sugar:

Internal cardiac, 0.153 External cardiac, 0.152 Pyloric, 0.147

The ratio of sugar production in the two parts of the stomach when flesh is added to the carbohydrate food — about 10 to 10.3 — need only to be compared with the ratio obtained when carbohydrate food alone is given — 10 to 17.6 — to see that the proteid protects the ptyalin from the acid in the pyloric end and permits the diastatic action to continue for an hour at least at a rate equal to that in the cardiac end. It should be remarked that the protection afforded by the proteid is really effective only in the relatively small pyloric portion of the stomach and to some extent on the surface of the cardiac portion. The greater mass of the food, lying in the fundus, undergoes uninterrupted amylolysis, not because the proteid protects the ptyalin, but because the food in this region is not mixed with the gastric juice.

**Change of starch into dextrin.** — Starch not changed to maltose in the stomach may be changed in considerable degree to dextrin. Inasmuch as dextrin is not directly fermentable, the amount of dextrin thus produced represents just so much carbohydrate food preserved from possible loss to the organism by fermentation. The amount of dextrin formed was roughly calculated in several cases as follows. One gram of the dried stomach contents was taken, about 100 c.c. water were added, and after one half hour the mixture was filtered through a weighed filter, on which the residue (starch and proteid) was several times washed, then dried, and residue and filter together weighed again. The loss of weight was taken to represent the soluble carbohydrates. From the total weight of soluble carbohydrates was deducted the weight of the sugar, and the remainder indicated the dextrin formed. The salts present were not determined. The dextrin thus roughly estimated varied in several instances between seventeen and forty per cent. The following example illustrates the relations between the various products in the different parts of the stomach:

| Aug. 1 | Amount taken. | Starch. | Dissolved. | Maltose. | Dextrin. |
|---|---|---|---|---|---|
| Internal cardiac | 1.0 | 0.354 | 0.646 | 0.250 | 0.396 |
| External cardiac | 1.0 | 0.441 | 0.559 | 0.237 | 0.322 |
| Pyloric | 1.0 | 0.687 | 0.313 | 0.135 | 0.178 |

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In all cases there was a large amount of dextrin in the internal part of the cardiac contents, to which the hydrochloric acid penetrates last. Evidently fermentation may proceed for a long time in this region unchecked by acidity. It is of obvious advantage, therefore, to have in this region a considerable amount of the starch ingested preserved to the organism by being changed to a form fermentable with difficulty or not at all.

## Discussion of Results.

In reviewing the observations here recorded on salivary digestion in the cardiac and pyloric ends of the stomach it is to be observed that invariably under normal conditions, when ordinary amounts of food are given, more sugar is produced in the cardiac than in the pyloric end. The conditions present in the cardiac end of the stomach are wholly in agreement with the contentions of observers who have urged that saliva may continue its chemical function for some time after being swallowed; the conditions present in the pyloric end, however, are very different, for the food there soon becomes acid and the action of the saliva stops. In 1880 von den Velden divided gastric digestion into two periods; in the first period, before free hydrochloric acid appears, the saliva swallowed with the food is still effective; in the second period, after free hydrochloric acid appears, only the pepsin continues activity. It is clear, however, from the results presented, that these two periods are different in the two ends of the stomach; in the pyloric end the first period is short, in the cardiac end it may last three or four times longer than it lasts in the pyloric end. In the early stages of digestion in the stomach, therefore, the cardiac end serves chiefly for salivary digestion; the pyloric end, after a brief course of salivary digestion, is thenceforth the seat of the strictly peptic changes. Later, as the cardiac contents become penetrated by gastric juice, diastatic activity ceases, and the stomach contents as a whole are subjected to the action of proteolytic ferments.

It is of interest to note that the results reported in this paper are in close agreement with the results obtained by Ellenberger and Hofmeister [1] in their observations on the horse and pig, and by Hohmeier [2] in his observations on the rat. The cardiac end of the stomach in

[1] Ellenberger and Hofmeister: Archiv für wissenschaftliche und praktische Thierheilkunde, 1884, vii, p. 6, and 1886, xii, p. 126.

[2] Hohmeier: Inaugural-Dissertation, Tübingen, 1901.

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the horse, the pig, and the rat is to a great extent lined with pavement epithelium and with "cardia glands," distinguished from the fundus glands in not having an acid secretion.[1] According to these observers this region becomes, in the absence of gastric secretion, the seat of prolonged amylolysis. That there is this similarity in salivary digestion between animals without acid secretion in the cardiac end and animals with free secretion there, indicates that the division of the stomach into two regions with functions chemically different during the early stages of gastric digestion is a general fact. The important agent in either case is the mechanical agent — the absence of peristalsis in the cardiac end and the consequent quiescence of the food in this region. As already pointed out (p. 401), the distinction between the active pyloric and the inactive cardiac end of the stomach has already been observed in many animals including man. It is altogether probable, therefore, that in man as in other animals the cardiac end serves chiefly for the action of ptyalin during the early stages of gastric digestion.

The recent observations of Müller and of Hensay, together with the results here presented, emphasize strongly the importance of mastication. Only by mastication is the food properly mixed with saliva and properly broken up so that all parts of it can be penetrated readily by the saliva. When the food has thus been thoroughly insalivated it will undergo to a great degree salivary digestion in the cardiac end of the stomach.

## Summary.

The evidence that the action of ptyalin is inhibited in the stomach soon after the ingestion of food is inconclusive. The support for this evidence from the commonly accepted accounts of mixing currents in the stomach is not well founded. Observations show that in many animals, including man, gastric peristalsis occurs only in the pyloric end of the stomach; the cardiac end remains undisturbed by the waves. Food in the pyloric end is soon mixed with the gastric secretions, but food in the cardiac end of the stomach is not mixed with the acid gastric juices for two hours or more, and in this region, therefore, during that time salivary digestion may go on undisturbed.

Examination of the dried contents of the pyloric and cardiac portions of the stomachs of cats, after carbohydrate food mixed with

[1] Oppel: Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere, erster Theil, Der Magen, Jena, 1896, pp. 240, 337, 346, 397.

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active saliva has been given, shows that the percentage of sugar present is about the same in the two portions at the end of a half hour, and at the end of an hour the cardiac portion contains about eighty per cent more sugar in unit volumes than the pyloric portion. The actual amount of sugar present in the fundus is relatively much greater than this ratio would indicate, for the fundus contains after an ordinary meal about five times as much food as the pyloric portion.

After an hour the ratio of the sugar percentages in the two parts of the stomach begins to approximate unity again. This change is probably due largely to diffusion of sugar from the fundus into the pyloric end, and to some extent to absorption.

The diffusion of the sugar does not to a marked degree remove the ptyalin from the food.

Position does not very notably affect the differences in sugar production between the two parts of the stomach, although with the fundus lower than the pyloric portion slightly more sugar is found in the fundus than when the opposite relation is maintained.

When liquid food is given, when small amounts of food are given, and when the stomach is massaged, sugar percentages in the two parts of the stomach are nearly the same.

Mixing proteid with carbohydrate food protects the ptyalin from the action of free hydrochloric acid in the relatively small pyloric part of the stomach and on the surface of the cardiac contents. The greater mass of the food, lying in the fundus, undergoes uninterrupted amylolysis, not because the proteid protects the ptyalin, but because the food in this region is not mixed with the gastric juice.

Much of the starch not changed to sugar is changed to dextrin, and thus, since dextrin is not readily fermented, the food is saved to the organism. The especial value of this process lies in the fact that it occurs to the greatest degree in the fundus, in which region the hydrochloric acid, inhibiting the action of many of the organized ferments, does not for some time make its appearance.

In the early stages of gastric digestion, if food has been properly masticated, the fundus serves chiefly for the action of the ptyalin; the pyloric portion, after a brief stage of salivary digestion, is thereafter the seat of strictly peptic changes. Later, after two hours or more, as the contents of the fundus become acid, the food in the stomach as a whole is subjected to the action of proteolytic fermentation.

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# NUCLEIN METABOLISM IN LYMPHATIC LEUKÆMIA.

BY YANDELL HENDERSON AND GASTON H. EDWARDS.

[*From the Physiological Laboratory of the Yale Medical School.*]

ALTHOUGH the metabolism of the nucleins has been the subject of a considerable number of investigations during the last few years, the net result has left much of uncertainty regarding the processes involved in the various forms of leucocytosis, physiological, experimental, and pathological. The first broad comparative study of the variations of metabolism involved in these conditions was that of Milroy and Malcolm.[1] On the basis of their own researches and those of previous investigators they drew a sharp distinction between forms of leucocytosis which previously (and on the basis merely of the similarities in the blood counts) had been regarded as essentially similar. They showed that while in the leucocytosis produced by the injection or ingestion of nuclein the increased number of the leucocytes in the blood is associated with an increased excretion of uric acid and phosphates (the latter in greater amount than could originate in the nuclein absorbed), in chronic leukæmia on the other hand the excretion of these substances is rather below the average for normal individuals. To these conclusions v. Moraczewski[2] has added the observation that in the cases of leukæmia studied by him there was a marked retention of nitrogen, phosphorus, chlorine, and calcium, and an alteration in the relative amounts of sodium and potassium excreted. In acute leukæmia, on the contrary, Magnus Levy[3] has found that the excretion of nitrogen, uric acid, and phosphates is enormously increased, coincident, however, with only a slight leucocytosis. Accordingly White and Hopkins,[4] in discussing the results of these investigators and adding observations made by themselves,

[1] MILROY and MALCOLM: Journal of physiology, 1898, xxiii, p. 232; and 1900, xxv, p. 105.

[2] v. MORACZEWSKI: Virchow's Archiv für pathologische Anatomie, 1898, cli, p. 22.

[3] LEVY: *Ibid.*, 1899, clii, p. 107.

[4] WHITE and HOPKINS: Journal of physiology, 1899, xxiv, p. 42.



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are led to the conclusion that there is no " necessary proportionality between the number of circulating leucocytes and the excretion of those products ($P_2O_5$ and alloxuric bodies) which result from the breakdown of nucleins."

Recently there has been under treatment in the public clinic of the Yale Medical School a case of lymphatic leukæmia which, through the kindness of Dr. O. T. Osborne,[1] we have been enabled to observe. As the clinical aspects of this case have already been discussed elsewhere no further description of them is needed here than to say that the patient was a male, sixty-four years of age, and presented a case of leukæmia typical of the purely lymphatic variety of slow development and progress. It was therefore especially suited to the study of metabolism in this form of leucocytosis.

Although no attempt was made to regulate the diet a careful record was kept, and it was found to vary only within very narrow limits, and to be almost nuclein free. It consisted of oatmeal, bread, potatoes, milk, butter, cheese, eggs, and occasionally beef, tea, coffee, and a small amount of whiskey. The urine was collected for twenty-four hours in bottles containing a few cubic centimetres of toluol, and was brought to the laboratory next day for analysis. Total nitrogen was estimated by the Kjeldahl-Gunning method; uric acid by Hopkins's method, weighing the crystals; phosphoric acid by titration with uranium acetate, using potassium ferrocyanide as indicator; total acidity and chlorides by the ordinary methods. In order to discover whether there might not be an elimination of phosphorus in organic combination a number of determinations were made of the total amount after evaporation of a sample of urine and ignition with sodium hydroxide and potassium nitrate. The results, however, agreed entirely with those obtained by the titration method. Allantoïn was looked for by evaporating 400 c.c. of urine to a syrup, adding a little strong acetic acid, and after several weeks examining for the crystals. The results were wholly negative. The unreliability of some, and the difficulties of all of the methods at present available for the estimation of the xanthin bases, and the fact that there is no reason to suppose that the relative amounts of uric acid and the other alloxuric bodies vary in any considerable degree dissuaded us from the attempt to determine this excretion. The urine was at all times wholly free from albumin or sugar and in all other respects entirely normal.

[1] OSBORNE, O. T.: American medicine, 1902, iv, p. 533.

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The results of observations extending over more than six months are given in the accompanying table, together with the calculated ratios of the principal excretives, and such other data as seem of importance. The significance of the figures is perhaps best seen in the charts showing their variations. The data presented show that in spite of the enormous leucocytosis (175000–380000 per cubic millimeter, of which 96 per cent were lymphocytes), and in spite of the alternations of great increase and equally marked diminution in the number of circulating corpuscles, the excretion of uric acid and phosphates was at no time excessive. The leucocytosis seems to be due not to a general increase in nuclein metabolism, but to a failure in the normal destructive processes. A diminution in the number of circulating leucocytes was accompanied by a considerable increase in uric acid excretion, although the actual amount excreted was small compared with the reduction in the number of circulating leucocytes. Their diminution was therefore probably due to an abstraction from the blood and storage, rather than to a destruction of corpuscles. This view is supported also by the changes observed in the size of the lymph glands.

More detailed consideration reveals two periods in which the course of metabolism was markedly different. During these the average daily amounts and ratios were as follows:

| October and November. | January to April. |
|---|---|
| N 12 grs. | N 9 grs. |
| Uric acid : N : : 1 : 23 | Uric acid : N : : 1 : 15 |
| $P_2O_5$ : N : : 1 : 8 | $P_2O_5$ : N : : 1 : 11 |
| Uric acid : $P_2O_5$ : : 1 : 3 | Uric acid : $P_2O_5$ : : 1 : 1.5 |

In the first period the total nitrogen was, if anything, slightly subnormal, the uric acid slightly above the normal, and the phosphates distinctly subnormal, — the normal ratio of $P_2O_5$ to nitrogen being about 1 to 5. Contrasted with these conditions the second period shows a distinct diminution in nitrogen excreted, a more than proportionate increase in the ratio of the uric acid to nitrogen (due to a slight increase in the absolute amount of uric acid), and a further great reduction in the ratio of phosphates to nitrogen in spite of the diminution in the latter. Taking the normal ratio of uric acid to

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TABLE OF OBSERVATIONS ON LYMPHATIC LEUKÆMIA.

| Date. | | Treatment.[1] | Analyses of Urine. | | | | | | | Ratios. | | | Blood Counts. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Vol. | Sp. gr. | Acidity as HCl. | Chlorides as NaCl. | Total N. | Uric acid. | $P_2O_5$. | $\frac{\text{Uric acid}}{\text{Total N}}$. | $\frac{P_2O_5}{\text{Total N}}$. | $\frac{\text{Uric acid}}{P_2O_5}$. | Whites. | Reds. |
| | | | c.c. | | grams. | grams. | grams. | grams. | grams. | | | | | |
| Oct. | 15 | Bone marrow. (Oct. 15 – Nov. 2) | 800 | 1020 | 1.57 | 7.6 | 9.84 | 0.438 | 1.51 | 1 : 22.5 | 1 : 6.5 | 1 : 3.5 | 175,000 | 2,719,000 |
| " | 17 | | 1150 | 1020 | 1.44 | 11.6 | 13.64 | 0.545 | 1.44 | 1 : 25.0 | 1 : 9.5 | 1 : 2.6 | | |
| " | 29 | | 1300 | 1015 | 1.53 | 14.6 | 9.97 | 0.425 | 1.00 | 1 : 23.7 | 1 : 10.1 | 1 : 2.4 | | |
| " | 31 | | 1350 | 1018 | 1.50 | 12.4 | 10.50 | 0.455 | 1.60 | 1 : 23.1 | 1 : 6.6 | 1 : 3.5 | | |
| Nov. | 2 | | 1100 | 1021 | 1.41 | 11.2 | 11.25 | 0.500 | 1.62 | 1 : 22.5 | 1 : 6.9 | 1 : 3.2 | 193,000 | 3,931,000 |
| " | 6 | Arsenic. (Nov. 6 – 11) | 1100 | 1022 | 1.76 | 10.0 | 13.90 | 0.455 | 1.60 | 1 : 30.5 | 1 : 8.7 | 1 : 3.5 | | |
| " | 8 | | 900 | 1026 | 1.80 | 9.8 | 13.77 | 0 560 | 1.23 | 1 : 24.6 | 1 : 11.2 | 1 : 2.2 | | |
| " | 11 | | 750 | 1018 | 1.60 | 8.4 | 10.36 | 0.343 | 0.98 | 1 : 30.2 | 1 : 10.6 | 1 : 2.9 | 203,000 | 3,296,000 |
| " | 14 | | 1000 | 1021 | 1.73 | 13.2 | 12.40 | 0.440 | 1 62 | 1 : 28.2 | 1 : 7.6 | 1 : 3.7 | | |
| " | 17 | | 1100 | 1020 | 1.50 | 15.2 | 10.76 | 0.445 | 1.23 | 1 : 24.2 | 1 : 8.7 | 1 : 2.8 | | |
| " | 19 | $NaHCO_3$ (Nov. 19 – 26) | 1300 | 1022 | 1.63 | 15.6 | 11.54 | 0.672 | 1.74 | 1 : 17.2 | 1 : 6.6 | 1 : 2.6 | | |
| " | 21 | | 1850 | 1019 | 1.67 | 21.0 | 12.20 | 0.620 | 1.42 | 1 : 19.7 | 1 : 8.6 | 1 : 2.3 | | |
| " | 24 | | 1100 | 1024 | 2.08 | 8.6 | 14.63 | 0.965 | 2.67 | 1 : 15.2 | 1 : 5.5 | 1 : 2.8 | | |
| " | 26 | | 800 | 1026 | 2.75 | 2.8 | 14.22 | 0.800 | 3.29 | 1 : 17.8 | 1 : 4 3 | 1 : 4.1 | 380,000 | 2,840,000 |

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| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dec. 4 | | 1300 | 1013 | 1 57 | 7.4 | 8.9 9 | 0.300 | 1.40 | 1 : 30.0 | 1 : 6.4 | 1 : 4.7 | | |
| " 8 | | 1400 | 1017 | 1.98 | 12.4 | 12.78 | 0.675 | 1.71 | 1 : 18.9 | 1 : 7.5 | 1 : 2.3 | | |
| " 12 | | 600 | 1024 | 1.92 | 6.6 | 9.18 | 0.555 | 0.98 | 1 : 16.5 | 1 : 9.4 | 1 : 1.8 | | |
| Jan. 7 | | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 256,000 | 3,662,000 |
| " 26 | | 550 | 1023 | 1.60 | 6.1 | 8.45 | 0.557 | 0.68 | 1 : 15.2 | 1 : 12.4 | 1 : 1.2 | | |
| " 28 | Bone marrow. | 500 | 1027 | 1.26 | 6.5 | 8.10 | 0.645 | 0.60 | 1 : 12.4 | 1 : 13.5 | 1 : 0.9 | | |
| " 31 | | 450 | 1025 | 1.39 | 4.6 | 7.78 | 0.635 | 0.66 | 1 : 12.2 | 1 : 11.8 | 1 : 1.0 | | |
| Feb. 15 | | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 380,000 | 4,060,000 |
| " 22 | | 525 | 1025 | 1.50 | 5 9 | 8.85 | 0.625 | 0.83 | 1 : 14.1 | 1 : 10.7 | 1 : 1.3 | | |
| " 24 | Nucleic acid. | 650 | 1024 | 1.56 | 6.3 | 10.51 | 0.560 | 0.98 | 1 : 18.8 | 1 : 10.7 | 1 : 1.7 | | |
| " 26 | | 750 | 1018 | 1.46 | 6.2 | 9.64 | 0.650 | 0.99 | 1 : 14.8 | 1 : 9.5 | 1 : 1.5 | | |
| " 28 | | 850 | 1023 | 2.11 | 6.6 | 11.32 | 0.685 | 1.12 | 1 : 16.5 | 1 : 10 1 | 1 : 1.6 | | |
| March 2 | | 575 | 1025 | 1.60 | 5.8 | 8.14 | 0.500. | 0.86 | 1 : 16.3 | 1 : 9.5 | 1 : 1.7 | 292,000 | 4,410,000 |
| " 5 | | 550 | 1025 | 2.03 | 6.1 | 8.85 | 0.530 | 0.74 | 1 : 16.7 | 1 : 12.0 | 1 : 1.4 | | |
| " 13 | | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 238,000 | 3,112,000 |
| April 23 | | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 68,000 | 3,616,000 |
| " 30 | | 1000 | 1019 | 2.49 | 8.2 | 10.15 | 0.752 | 1.10 | 1 : 14.8 | 1 : 9.2 | 1 : 1.5 | | |
| May 1 | | 1300 | 1014 | 2.69 | 7.8 | 9.84 | 0.725 | 0.90 | 1 : 13.6 | 1 : 10.9 | 1 : 1.2 | | |

[1] The treatment is indicated for the sake of completeness, but is not concerned in the data or conclusions. We are indebted to Messrs. Park Davis & Co. for the "nucleic acid" used.

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$P_2O_5$ to be 1 to 4 or 5, the tendency to a progressive diminution in the excretion of phosphates is strikingly illustrated by the figures presented above. Although the diet was not regulated it remained the same in kind, and there was no reason to believe that the amount was diminished during the second as compared with the first period, — certainly not to the extent suggested by the nitrogen eliminated. The explanation of the small nitrogen of the second period is probably to be found in a nitrogen retention, a general diminution of proteid metabolism. Furthermore the amounts and the ratios of the phosphates show that of this excretion also there was a retention. Indeed, in this case the retention was much more distinctly indicated and was much greater relatively than with the nitrogen. That this retention was not due to a diminished breaking down of nuclear material during the second as compared with the first period, is shown by the fact that there was a coincident slight increase in uric acid output. Nor can the low phosphorus output through the urine be explained by an increased excretion through the intestine. The ratio of $P_2O_5$ to nitrogen in the fæces was indeed so high as to suggest such an avenue of excretion, but the absolute amounts of $P_2O_5$ in the fæces were insufficient to account for more than a part of the urinary deficiency.

ANALYSES OF FÆCES OF SEVEN-DAY PERIODS.

| Date. | Dry solids. | Total N. | Total $P_2O_5$. | N. per diem. | $P_2O_5$ per diem. |
|---|---|---|---|---|---|
| | grams. | grams. | grams. | grams. | grams. |
| Jan. 24–31 | 80.0 | 4.27 | 6.76 | 0.61 | 0.97 |
| Feb. 21–28 | 76.0 | 3.53 | 5.17 | 0.51 | 0.74 |

In the chlorides also there was strong evidence of a retention during the second period; and the small volume of the urine and the occurrence of œdema in the ankles during the latter half of February suggest a considerable retention of water. The contrast afforded by the conditions existing during October and November with those during January and thereafter is the more interesting because the former are essentially similar to the conditions observed by Milroy and Malcolm, and the latter are equally the counterpart of those found by v. Moraczewski. The differences in the results of these observers are probably to be assigned, therefore, to the different stages to which the leukæmic conditions had developed when they came under study.

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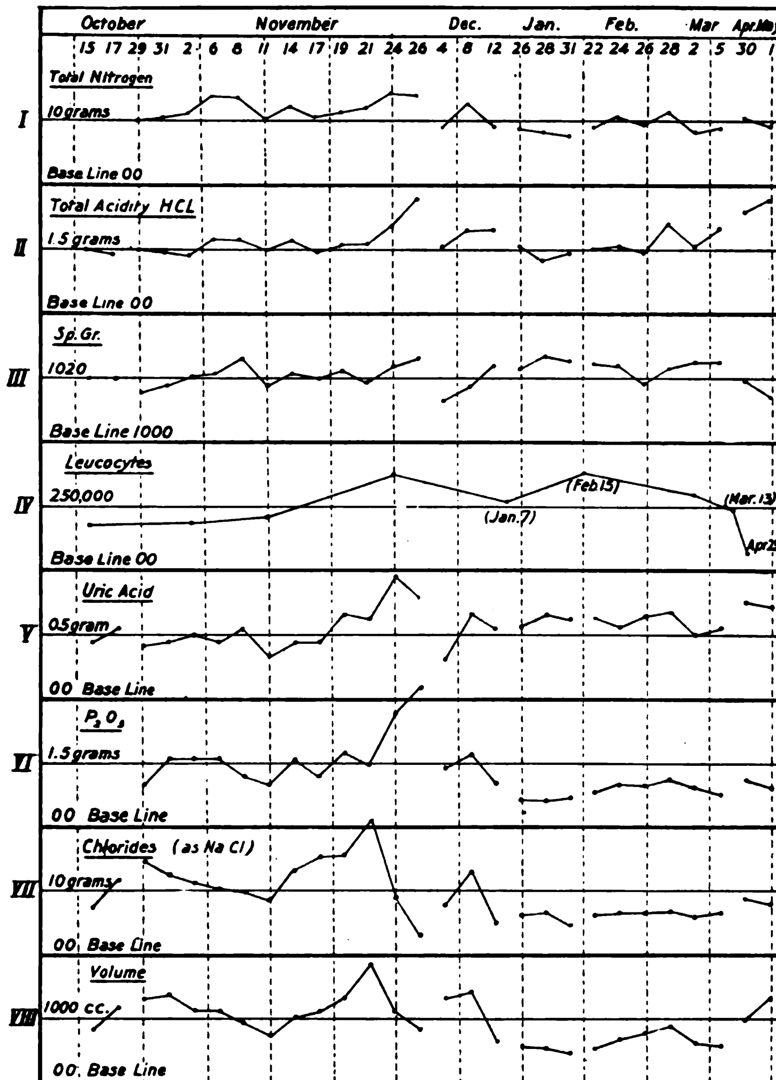
CHARTS SHOWING THE RESULTS OF URINARY ANALYSES AND BLOOD COUNTS.




The points indicated in these charts are: —

1. The curves for total nitrogen and acidity are very similar. If they were completely superimposable an excretion in the uniform ratio of 10: 1.5 would be demonstrated.

2. The curves for uric acid and $P_2O_5$ are very similar during the "first period" (see text), and together with the sp. gr. are approximately parallel to total nitrogen. For the "second period" there is no such parallelism.

3. The curves for chlorides and volume are strikingly similar, but show no relation to those before mentioned.

4. Except during the later days of November the number of leucocytes bears no apparent relation to any of these curves.

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Referring to the charts, it will be seen that throughout both periods the total acidity was closely parallel to the nitrogen, which accords with the view that the total acidity is chiefly an expression of the $SO_3$ resulting from the proteid metabolism. Accepting this idea, the parallelism in the two curves is an indication of the lack of any marked disturbance in proteid metabolism. During the first period the specific gravity ran quite closely parallel to the total nitrogen (urea), without regard to the volume, while during the second period the specific gravity shows no relation to either volume or nitrogen. The chlorides varied more than any other excretive (from 2.8 to 21.0 grams per day), yet throughout both periods was closely paralleled by the figures for the volume of the urine. On the other hand, these two curves exhibit no relation to those of nitrogen and acidity. During the first period, as already stated, the uric acid and phosphates were closely parallel, and their variations when studied in connection with the curves for the number of circulating leucocytes and the excretion of chlorides (especially during the time between the eleventh of November and the fourth of December) are very suggestive. They seem to show that during this time there occurred (1) a marked increase in nuclear break-down, followed by a return to the usual rate; (2) an increase in the number of circulating leucocytes (probably thrown out of the glands where they had been stored) followed by a diminution in their number; and (3) coincident with these changes, or slightly preceding them, a considerable increase in the excretion of chlorides followed an immense diminution.

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# THE EFFECT OF DIURETICS, NEPHRITIC POISONS, AND OTHER AGENCIES ON THE CHLORIDES OF THE URINE.

By TORALD SOLLMANN.

[*From the Pharmacological Laboratory of Western Reserve University, Cleveland, Ohio.*]

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## I. Introductory.

IN a previous paper[1] I pointed out the importance of the phenomenon of chloride retention to the theory of the mechanism of urine secretion, and I discussed there the data which we possessed at that time. It was seen that these were sufficient to define the problem more or less sharply, but that they did not justify any definite conclusions. In the present investigations I have attempted to approach the problem, in the first place, by studying the effect of various classes of diuretic and nephritic agents upon chloride excretion. After the research had been planned in detail there appeared a series of papers from Filehne's laboratory[2] and a research by Loewi[3] which dealt with some of these problems. These were, however, to some extent mutually contradictory, and covered only a part of the questions which I intended to investigate. I therefore carried out the research as planned, with the result that the cause of the contradictory results was cleared up, and that the facts are now sufficiently numerous for safe generalizations.

## II. Methods.

**1. Injection experiments.** — The experiments were made exclusively on dogs. As it was my purpose to investigate as many conditions as possible, no precautions were taken to keep the animals on a uniform diet. Some urines were accordingly rich in chloride, others were poor in this ion. This did not influence the results. The animals received a large dose of morphine, and were kept under a light ether anæsthesia throughout the experiments. In Experiments VIII to XXIX, both vagi were divided to abolish any inhibitory effect of the vagi on urine secretion, such as is claimed by Corin, and confirmed by the experiments of Anten.[4] The ureters were exposed by a small incision through the linea alba, and narrow glass cannulæ were introduced near the bladder. The urine was collected continuously, the collecting vessel being changed every ten to twenty minutes. The injections were made into the femoral vein. The fluids were heated to

[1] Sollmann, T.: This journal, 1902, viii, p. 155.

[2] Filehne, W.: Biberfeld, Ruschhaupt, Pototzky, and Ercklentz: Archiv für die gesammte Physiologie, 1902, xci, p. 565.

[3] Loewi, O.: Archiv für experimentelle Pathologie, 1902, xlviii, p. 410.

[4] Anten, H.: Archives internationales de Pharmacodynamie, 1901, viii, p. 455.

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± 38° C., and injected in two to four minutes, usually an hour apart. The first injection was of 35 c.c. per kg., the succeeding injections each usually of 25 c.c. per kg. Three or four injections were made. The urine was collected for about an hour before the first injection. It was found that the composition of the bladder urine bears no relation to that secreted after the operation.

The saline diuretics were used in the uniform strength of $\frac{m}{7}$. The following solutions were employed:[1] —

*Sodium salts.* — Acetate, 1.94 per cent crystals; ferrocyanide, 7.43 per cent crystals; iodide, 2.16 per cent; phosphate ($Na_2HPO_4$), 5.10 per cent crystals; sulphate, 4.6 per cent crystals; sulphocyanide, 1.16 per cent.

*Non-electrolytes.* — Glucose (C. P. Dextrose), 2.57 per cent; urea, 0.886 per cent, in water or in $\frac{m}{7}$ sulphate.

*Non-saline diuretics.* — Alcohol, 3 per cent in water or in $\frac{m}{7}$ sulphate; juniper oil, 0.4 per cent, and alcohol 1.6 per cent, in sulphate; caffein, 0.04 per cent of citrated caffein in $\frac{m}{7}$ sulphate; phlorhizin, 0.4 per cent in water or in $\frac{m}{7}$ sulphate; methylene blue, 0.5 per cent in $\frac{m}{7}$ sulphate. The citrated caffein was also used hypodermically in 1 per cent solution.

*Nephritic agents.* — These will be discussed in the text.

**2. Methods of analysis.** — The *chlorides* were determined by evaporating the urine (usually 5 to 20 c.c.) with about half a gram $Na_2CO_3$, carbonizing, and fusing with sufficient $NaNO_3$; dissolving, neutralizing with $HNO_3$ and titrating with $AgNO_3$ (1 c.c. = 1 mg. NaCl), using chromate as indicator.

In the presence of iodides, the method of Salkowski[2] was used.

Neubauer and Vogel[3] quote this paper of Salkowski as reference for the statement that the method can also be used for bromides. The quotation is incorrect and the method not applicable: 10 c.c. of NaBr solution require before treatment, 10 c.c. of $\frac{n}{1}$ $AgNO_3$; after treatment (when they should require no $AgNO_3$) they use 7.3 c.c. in one test, 8.0 c.c. in another.

In a few experiments, in which the freezing point and nitrogen were determined, this was done by the method of Beckmann and of Kjeldahl.

[1] For a 10 per cent solution, 10 grams of the salt are dissolved in 90 grams of distilled water.

[2] SALKOWSKI, E.: Archiv für die gesammte Physiologie, 1872, vi, p. 214.

[3] NEUBAUER and VOGEL (H. HUPPERT): Analyse des Harnes, 10th edition, Wiesbaden, 1898, p. 713.

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## III. Results of Injection Experiments.

**1. Effect of sodium sulphate injection on chlorides, Δ, and nitrogen.** — Three preliminary experiments were made to ascertain whether it

| | Diuresis in 10 m. | Δ. | N. | Cl. | Δ×c.c. | Mg. in 10 m. N. | Mg. in 10 m. Cl. | $\frac{N}{Cl}$. |
|---|---|---|---|---|---|---|---|---|
| **Experiment I.** | | | | | | | | |
| | | | per cent. | per cent. | | | | |
| Bladder urine . . . . | .... | 0.790 | 0.798 | 0.243 | .... | .... | .... | 3.3 |
| Before injection . . . | 0.2 | .... | .... | .... | .... | .... | .... | .. |
| After injection: | | | | | | | | |
| 0 to 17 m. . . . . . | 14.7 | 0.775 | 0.127 | 0.035 | 11.39 | 18.73 | 5.15 | 3.6 |
| 17 to 58 m. . . . . | 11.4 | 0.790 | 0.094 | 0.020 | 9.01 | 10.69 | 2.28 | 4.7 |
| 58 m. to 1 hr. 48 m. . | 10.0 | 0.765 | 0.120 | 0.060 | 7.65 | 12.04 | 6.00 | 2.0 |
| 1 h. 48 m. to 2 h. 34 m. | 10.9 | 0.640 | 0.123 | 0.055 | 7.18 | 13.43 | 6.00 | 2.2 |
| 2 h. 34 m. to end . . | .... | .... | 0.119 | .... | .... | .... | .... | .. |
| **Experiment II.** | | | | | | | | |
| Before injection . . . | 2.0 | .... | 0.527 | .... | .... | 10.55 | .... | .. |
| After injection: | | | | | | | | |
| 0–21 m. . . . . . . | 11.4 | 0.895 | 0.112 | 0.040 | 10.12 | 12.77 | 4.56 | 2.8 |
| End . . . . . . . . | .... | .... | 0.063 | .... | .... | .... | .... | .. |
| **Experiment III.** | | | | | | | | |
| Bladder urine . . . . | .... | .... | 3.664 | .... | .... | 21.98 | .... | .. |
| Before injection . . . | 0.6 | .... | .... | .... | .... | .... | .... | .. |
| After injection: | | | | | | | | |
| 26 to 38 m. . . . . | 8.9 | 0.740 | 0.140 | 0.040 | 6.59 | 12.46 | 3.56 | 3.5 |
| 38 to 60 m. . . . . | 22.7 | 0.765 | 0.112 | 0.020 | 17.36 | 25.42 | 4.54 | 5.6 |
| 60 m. to 1 hr. 35 m. . | 22.7 | 0.740 | 0.118 | 0.020 | 16.80 | 26.79 | 4.54 | 5.9 |
| 1 h. 35 m. to 2 h. 29 m. | 25.0 | 0.660 | 0.122 | 0.020 | 16.50 | 30.60 | 5.00 | 6.1 |
| 2 h. 29 m. to 3 h. 14 m. | 22.7 | 0.740 | 0.158 | 0.020 | 16.50 | 35.85 | 4.54 | 7.9 |
| 3 h. 14 m. to 4 h. 04 m. | 14.7 | 0.790 | 0.193 | 0.035 | 11.61 | 28.40 | 5.15 | 5.8 |
| 4 h. 04 m. to 4 h. 45 m. | 12.5 | 0.835 | 0.231 | 0.033 | 10.44 | 28.94 | 4.13 | 7.0 |

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would be necessary to determine the changes in nitrogen and the freezing-point, as well as the chlorides.

The three experiments gave very uniform results, the urinary constituents during active diuresis varying as follows: —

*Depression of freezing-point:* 0.640 to 0.790° C. (This factor varied between 0.85 and 1.30 in Experiments VI and VII, in which 35 c.c. per kg. $Na_2SO_4$, 25 c.c. urea, and 25 c.c. glucose were injected an hour apart.) Per cent **N**, 0.0938 to 0.1232; per cent **NaCl**, 0.020 to 0.40; $\frac{\mathbf{N}}{\mathbf{NaCl}}$, 2.8 to 7.9.

The increased diuresis, as usual, tends to lessen the concentration of the urinary constituents, whilst it increases their absolute quantity. The per cent of chlorine and the freezing-point are but little affected by the degree of diuresis. The diuresis reaches its maximum in twenty to sixty minutes, and is still quite perceptible in five hours.

The nitrogen and chlorine change generally in the same direction, but by no means in the same proportion, the factor $\frac{N}{NaCl}$ varying between 2.0 and 7.9; the difference is seen very strikingly in the last two urines of Experiment III. The factor $\frac{N}{NaCl}$ is independent of the diuresis, and does not vary inversely to the diuresis, as would be demanded by the reabsorption theory.

Since the nitrogen and $\Delta$ vary quite independently of the chlorine, it is evident that they are controlled in part by other factors, which would complicate the problem. I deemed it better, therefore, to neglect them in the further experiments.

It was noticed incidentally that the depth of the color of the urines varied in the same direction as the per cent of nitrogen, so that the two would seem to be excreted by the same mechanism.

2. **Saline diuretics which lower the per cent of chlorine in the urine.** —This class comprises solutions of the acetate, ferrocyanide, phosphate, and sulphate of sodium; as also urea, glucose, and water. With the exception of the very small diuresis produced by the last substance, the phenomena are exactly alike in all cases.

The diuresis sets in very shortly after the injection. The per cent of chlorine falls at the same time to a very low figure (to less than 0.1 per cent, mean about 0.020 per cent). The chlorine is usually the lower, the poorer the original urine was in chlorides. The fall of the

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per cent of chlorine is entirely independent of the diuresis in quantity and in duration. The low content in chlorine always persists with little change for an hour, whilst the diuresis becomes very much less. If further injections are made the chlorine remains low or is lowered still further. The absolute amount of chlorine is uniformly increased by a considerable amount, varying with the diuresis.

The following abstracts of the experiments will illustrate these conclusions: —

*Sodium sulphate.* — Twelve experiments were made, the injections ranging from 25 to 75 c.c. per kg. These reduced the per cent of NaCl (from 0.019–0.555, mean 0.150) to 0.15–0.050, mean 0.018. Examples: —

| | Minutes after injection. | Diuresis in 10 min. | NaCl. | Total quantity of NaCl in 10 min. |
|---|---|---|---|---|
| **Experiment V.** | | | | |
| | | c.c. | per cent. | mgms. |
| Before injection . . . . . . . | .... | 0.75 | 0.280 | 2.10 |
| Injection of 35 c.c. per kg. of $SO_4$ | 0–15 | 8.0 | **0.015** | 1.20 |
| | 15–30 | 22.0 | 0.015 | 3.30 |
| | 30–45 | 13.0 | 0.015 | 1.95 |
| **Experiment VI.** | | | | |
| Before injection . . . . . . . | .... | 1.0 | 0.150 | 1.50 |
| Injection of 35 c.c. per kg. . . | 0–15 | 27.0 | **0.020** | 5.40 |
| | 30–45 | 12.0 | 0.015 | 1.80 |
| **Experiment XI.** | | | | |
| Before injection . . . . . . . | .... | 5.0 | 0.019 | 0.95 |
| Injection of 35 c.c. per kg. . . | 20–40 | 13.0 | **0.017** | 2.20 |
| | 40–60 | 17.5 | 0.021 | 3.65 |

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| Experiment XV.[1] | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| Before injection . . . . . . . | .... | 6.0 | 0 555 | 33.00 |
| Injection of 35 c.c. per kg. . . | 0–20 | 36 0 | **0.110** | 39.90 |
| | 20–60 | 18.0 | 0.118 | 21 24 |
| | 60–90 | 5.3 | 0.050 | 2.65 |
| **Experiment XVI.** | | | | |
| Before injection . . . . . . . | .... | 1.2 | 0.080 | 0.96 |
| Injection of 35 c.c. per kg. . . | 0–20 | 37.5 | **0.021** | 7.88 |
| | 20–40 | 23 0 | 0.016 | 3.58 |

*Sodium phosphate.* — Three experiments, with injections of 25 to 35 c.c. per kg. The per cent of NaCl is reduced (from 0.020–0.160) to 0.017–0.056; the absolute amount per 10 min. is increased (from 0.40–2.40) to 1.10–11.20 mg. Example: —

| Experiment XVIII.[1] | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| Before injection . . . . . . . | .... | 0.25 | 0.160 | 0.40 |
| Injection of 35 c.c. per kg. of $Na_2HPO_4$ . . . . . . . . | 0–20 | 18.5 | **0.045** | 8.35 |
| | 20–40 | 14.0 | 0.080 | 11.20 |
| | 40–60 | 5.5 | 0.063 | 3.46 |
| | 60–80 | 2.2 | 0 056 | 1.25 |

[1] The columns in these tables correspond to those of Experiments V to XI on the preceding page.

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*Sodium ferrocyanide.* — Two experiments, with injections of 25 and 35 c.c. per kg.

| Experiment XXVII.[1] | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| 35 c.c. per kg. of 3% alcohol in water; 25 c.c. per kg. $Na_2SO_4$[2] | .... | 9.0 | 0.019 | 1.71 |
| 25 c.c. per kg. $Na_4Fe(CN)_6$ . . | 0–10 | 25.0 | **0.025** | 6.25 |
| | 20–40 | 22.5 | 0.016 | 3.60 |
| | 60–80 | 12.5 | 0.027 | 3.38 |
| **Experiment XXVIII.** | | | | |
| Original urine . . . . . . . . | .... | 16 | 0.704 | 11.44 |
| 35 c.c. per kg. $Na_4Fe(CN)_6$ . . | 0–10 | 33.0 | **0.089** | 29.37 |
| | 10–20 | 24.0 | 0.038 | 9.12 |
| | 20–40 | 16.0 | 0.030 | 4.80 |

[1] The columns in these tables correspond to those of Experiments V to XI on page 430.
[2] Injections made an hour apart, before the injection of the ferrocyanide.

*Sodium acetate.* — Two experiments: the diuresis is quite small, but the effect on the per cent of chlorine is the same.

| Experiment XI. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| 35 c.c. per kg. $Na_2SO_4$ . . . . | .... | 17.5 | 0.021 | 3.65 |
| 25 c.c. per kg. $NaC_2H_3O_2$ . . . | 0-20 | 20.5 | **0.015** | 3.10 |
| | 20–40 | 13.0 | 0.022 | 2.85 |
| | 40–60 | 6.0 | 0.020 | 1.20 |

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| Experiment XVII. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| Original urine . . . . . . . . | .... | 1 6 | 0.070 | 1.12 |
| 35 c.c. per kg. $NaC_2H_3O_2$ . . . | 0–20 | 2.0 | **0.065** | 1 30 |
| | 20–40 | 4.3 | 0.044 | 1.87 |

*Urea.* — This substance, dissolved in water (three experiments) or in sulphate (one experiment), reduces the per cent of NaCl (from 0.015–0.055) to 0.012–0.046. Example: —

| Experiment VI. | | | |
|---|---|---|---|
| | Time. | Diuresis. | Per cent. |
| 35 c c. per kg. $Na_2SO_4$ . . . . | .... | 14.0 | 0.015 |
| 25 c.c. per kg. urea in water . . | 0–15 | 18.0 | **0.015** |
| | 30–45 | 12.0 | 0.012 |

The following experiment illustrates how a urine of very low chlorine content may show a slight temporary increase. This was occasionally seen with other salts, but was always negligibly small; it is probably to be explained by the theory of Cushny, *i. e.* by a lesser reabsorption of chlorine due to the diuresis.

| Experiment XXIV. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| $Na_2SO_4$, cantharidin . . . . | .... | 11.0 | 0.027 | 2.97 |
| 25 c.c. per kg. urea in $Na_2SO_4$ . | 0–10 | 36.0 | **0.046** | 16.56 |
| | 10–30 | 6.5 | 0.017 | 1.10 |

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*Dextrose.* — Three experiments with injections of 25 or 35 c.c. per kg. The per cent of NaCl is reduced (from 0.012–0.256) to 0.012–0.061. Example: —

| Experiment XIX. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| Before injection . . . . . . . | .... | 2.0 | 0.256 | 5.12 |
| 35 c.c. per kg. of glucose . . . | 0–20 | 10.0 | **0.082** | 8.20 |
| | 20–40 | 5.0 | 0.061 | 3.05 |
| | 40–60 | 1.8 | 0.103 | 1.80 |

*Water.* — One experiment: this reduces the per cent of chlorine in the same way as salts, but as it causes no diuresis, the absolute amount is also reduced.

| Experiment XXV. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| Watery alcohol, $Na_2SO_4$ . . . | .... | 15.0 | 0.036 | 5.40 |
| 25 c.c. per kg. of water . . . . | 0–15 | 16.5 | **0.029** | 4.79 |
| | 15–30 | 10.0 | 0 029 | 2.90 |

**3. Does the injection of the salts stimulate the retention of chlorides?** — To answer this question, the chlorine content of the urine was compared after injection of 0.5 per cent sodium chloride, dissolved in water and in 2.3 per cent sodium sulphate crystals. Three animals were used, the watery solution being injected first in two of these experiments, last in the other. There was no difference in the per cent, sodium chloride in water reducing the per cent in urine (from

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0.050–0.380) to 0.025–0.180; sodium chloride in sodium sulphate reducing the per cent in urine (from 0.029–0.180) to 0.020–0.045. The diminution of the chlorine is, therefore, not due to the presence of a foreign salt, but to the dilution of the blood. However, the total amount of chlorine is greater when sulphate is injected, on account of the greater diuresis. Example: —

EXPERIMENT XII.

| | Time. | Diuresis. | NaCl. | NaCl. |
|---|---|---|---|---|
| | | | per cent. | mg. |
| Before injection . . . . . . . | .... | 1.5 | 0.050 | 0.75 |
| 35 c.c. kg. 0.5% NaCl . . . . | 0–20 | 2.5 | 0.060 | 1.50 |
| | 20–60 | 1.9 | 0.029 | 0.55 |
| 35 c.c. kg. { 0.5 NaCl, 2.3 $Na_2SO_4$ } . . | 0–20 | 5.0 | 0.021 | 1.05 |
| | 20–40 | 15.0 | 0.023 | 3.45 |
| | 40–60 | 17.0 | 0.038 | 6.31 |
| | 60–80 | 2.0 | 0.033 | 0.66 |

**4. Saline diuretics which do not cause chlorine retention.** — Under this heading come: sodium nitrate, sulphocyanide, and iodide (and presumably also the bromide; but this was not tested, for want of a suitable method). These salts differ from the preceding, not merely by failure to cause chlorine retention, but they even increase the per cent of chlorine greatly if this has been artificially lowered. It is also remarkable that the injected iodide reacts toward sulphate injection just like the chloride, and that these ions were excreted (in the conditions of the experiment) in approximately equimolecular ratio. This was not investigated in connection with the other ions. Since Loewi[1] had already demonstrated the effect of the nitrate ion, I contented myself with a former experiment, already quoted in part[2] and which is inserted here somewhat more in detail, in addition to the experiments made with the other ions mentioned.

[1] LOEWI O.: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 410.

[2] SOLLMANN T.: This journal, 1902, viii, p. 155.

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*Nitrate experiment.* — Dog of 16 kg., injection of 75 c.c. per kg. of 1.23 per cent $NaNO_3$ solution (Δ 0.481), in 10 min.

URINE AFTER INJECTION.

| Time after injection. | Quantity in 10 min. | Δ. | NaCl. | $NaNO_3$. | $Na_2SO_4$. | Total solids. |
|---|---|---|---|---|---|---|
| | | | per cent. | per cent. | per cent. | per cent. |
| 10 min. . . | 23 | 1.357 | **0.40** | 1.481 | 0.217 | 4 193 |
| 24 min. . . | 20 | 1.042 | 0 38 | 1.572 | 0 067 | 2.780 |
| 42 min. . . | 27 | 1.110 | 0.37 | 1.679 | 0.064 | 2.570 |
| 1 hr. . . . | 25 | 1.117 | 0.35 | 1.836 | 0.051 | 2.910 |
| 1¾ hr. . . | 15⅘ | 1.102 | 0.28 | 1.631 | 0.079 | 2.580 |
| 2½ hrs. . . | 10 | 1.178 | 0.26 | 1.707 | 0.096 | 2.640 |
| 6 hrs. . . | 2¼ | 1.183 | 0.24 | 1.738 | .... | 3.745 |

SERUM.

| | Δ. | NaCl. | $NaNO_3$. | $Na_2SO_4$. | Serum in blood. |
|---|---|---|---|---|---|
| | | per cent. | per cent. | per cent. | per cent. |
| Before injection . | 0 616 | .... | 0.023 | 0.159 | 56.22 |
| After injection: | | | | | |
| 1 min. . . . . | 0.595 | 0.37 | 0.261 | 0.131 | 72.22 |
| 15 min. . . . . | 0.605 | **0.38** | 0.219 | none | 65 96 |
| 1 hr. . . . . | 0.605 | 0.43 | 0.122 | trace | 61.58 |
| 2½ hrs. . . . . | 0.596 | 0.48 | 0 146 | 0.023 | 58.10 |

It is seen that the urine after nitrate injection has a high per cent of chlorine, slightly higher than that of the serum.

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*Sulphocyanide :—*

| | Time. | Diuresis. | NaCl. | NaCl. |
|---|---|---|---|---|
| **Experiment XXVI.** | | | | |
| | | | per cent. | mg. |
| Original urine . . . . . . . . . . . | .... | 8.0 | 0.300 | 24.00 |
| Aqueous alcohol, $Na_2SO_4$, $Na_4Fe(CN)_6$ | .... | 12.5 | 0.027 | 3.38 |
| 25 c.c. per kg. NaSCN . . . . . . . | 0–10 | 25.0 | 0 278 | 97.30 |
| | 10–30 | 13.5 | 0.115 | 15.33 |
| | 30–50 | 10 0 | 0.054 | 0.54 |
| **Experiment XXVIII.** | | | | |
| Original urine . . . . . . . . . . . | .... | 1 6 | 0.704 | 11.44 |
| $Na_4Fe(CN)_6$ . . . . . . . . . . . | .... | 16.0 | 0.030 | 4.80 |
| 25 c c. per kg. NaSCN . . . . . . . | 0–20 | 12.5 | **0.227** | 26.11 |
| | 20–40 | 7.5 | 0.110 | 8.25 |
| | 40–60 | 6.0 | 0.122 | 7.32 |
| 25 c.c per kg. NaSCN . . . . . . . | 0–20 | 6.0 | **0.277** | 16.62 |
| | 20–40 | 4.0 | 0.159 | 6.35 |
| | 40–60 | 2.8 | 0.300 | 8.25 |

The sulphocyanide has raised the chlorine of the urine (from 0.030) to 0.300 per cent.

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*Iodide:* —

| | Time. | Diuresis. | NaCl. | NaI. | Equivalents.[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | NaCl. | NaI. | NaCl + NaI. | NaCl × c.c. | NaCl + NaI × c.c. | $\frac{NaI}{NaCl}$ |
| **Experiment XIV.** | | | | | | | | | | |
| | | | p. cent. | p. cent. | | | | | | |
| $Na_2SO_4$ . . . . | 20–30 | 33.0 | 0.027 | 0.0 | 0.562 | .... | 0.562 | 18.6 | 18.6 | .... |
| 25 c.c. kg. NaI . | 0–10 | 38.0 | 0.106 | 0.245 | 1.813 | 1.619 | 3.432 | 68.9 | 130.3 | 0.89 |
| | 10–20 | 31.0 | 0.100 | 0.243 | 1.710 | 1.606 | 3.316 | 53.0 | 102.7 | 0.93 |
| | 20–35 | 20.0 | 0.133 | 0.212 | 2.280 | 1.401 | 3.681 | 45.6 | 73.6 | 0.61 |
| 25 c.c. kg. phlorhizin in water . | 0–20 | 21.0 | 0.060 | 0.064 | 1.026 | 0.423 | 1.449 | 21.5 | 30.4 | 0.41 |
| | 20–40 | 13.5 | 0.096 | 0.094 | 1.642 | 0.621 | 2.263 | 22.1 | 30.5 | 0.38 |
| **Experiment XV.** | | | | | | | | | | |
| $Na_2SO_4$ . . . . | 60–90 | 6.0 | 0.050 | 0.0 | 0.860 | .... | 0.860 | 5.2 | 5.2 | .. |
| 25 c.c. kg. NaI . . | 0–20 | 12.5 | 0.248 | 0.773 | 4.241 | 5.110 | 9.351 | 53.1 | 116.9 | 1.25 |
| | 20–40 | 2.0 | 0.200 | .... | 3.420 | ... | .... | 6.8 | .... | .. |
| | 40–60 | 0.0 | .... | .... | .... | .... | .... | .. | .... | .. |
| 25 c.c. kg. $Na_2SO_4$ | 0–20 | 11.5 | 0.027 | 0.018 | 0.562 | 0.119 | 0.680 | 6.5 | 7.3 | 0.21 |
| | 20–40 | 12.5 | 0.022 | 0.020 | 0.376 | 0.132 | 0.508 | 4.7 | 6.3 | 0.35 |
| | 40–60 | 8.5 | 0.021 | 0.092 | 0.359 | 0.608 | 0.967 | 5.1 | 8.2 | 1.69 |

[1] $\frac{\text{Per cent} \times 100}{\text{molecular weight}}$ equals, for NaCl, per cent × 17.1; for NaI, per cent × 6.61.

Sodium iodide (two experiments) raises the per cent of chlorine in the urine. With the quantities which were used, the chlorine and iodine are excreted in approximately equimolecular proportion, but not quite so. Injection of sulphate or water cuts down the iodine in the same way as it does the chlorine.

**5. Effect of other diuretic agents.** — Alcohol, oil of juniper, ether, methylene blue, caffein citrate, or phlorhizin have no effect on the per cent of chlorine, beyond that of the vehicle in which they are administered.

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*Alcohol.* — Given intravenously in 3 per cent solution, in water (two experiments) or in $\frac{m}{7}$ $Na_2SO_4$ (one experiment), in dose of 25 and 35 c.c. per kg. When given in water there is much less diuresis, so that the total excretion of chlorine may also be diminished. Example: —

| Experiment XXVII. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | NaCl. | NaCl. |
| | | | per cent. | mg. |
| Before injection . . . . . . . | .... | 8.00 | 0.300 | 24.00 |
| 35 c.c. per kg. of 3% alcohol in water . . . . . . . . . . | 0–20 | 5.00 | 0.103 | 5.15 |
| | 20–40 | 3.25 | 0.043 | 1.40 |
| 25 c.c. per kg. of $Na_2SO_4$ . . . | 0–10 | 64.00 | 0.092 | 29.44 |

*Juniper.* — A mixture of 0.4 per cent juniper oil and 1.6 per cent of alcohol in $\frac{m}{7}$ $Na_2SO_4$ is given intravenously in the dose of 35 c.c. per kg.

| Experiment XVII. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | Per cent. | Mg. |
| $NaC_2H_3O_2$; alcohol in $Na_2SO_4$. | .... | 17.0 | 0.017 | 2.89 |
| Juniper mixture . . . . . . . | 0–20 | 40.0 | 0.019 | 7.60 |
| | 20–40 | 32.5 | 0.019 | 6.18 |

*Ether.* — This was injected hypodermically; 10 c.c. per kg., divided into 6 doses, being distributed over 50 minutes, when the animal died.

| Experiment XXV. | | | |
|---|---|---|---|
| | Time. | Diuresis. | Per cent. |
| Alcohol in water, $Na_2SO_4$; water, $Na_2SO_4$; ether | 0-10 | 32.0 | 0.015 |
| | 40–50 | 30.0 | 0.012 |

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*Methylene blue.* — 25 c.c. per kg. of a 0.5 per cent solution in $\frac{m}{7}$ $Na_2SO_4$ was injected intravenously. Two animals died, with anuria and strong convulsions, within 20 minutes after the injection. The following succeeded: —

| Experiment XVI. | | | |
|---|---|---|---|
| | Time. | Diuresis. | Per cent. |
| $Na_2SO_4$, phlorhizin, caffein . . | .... | 2.5 | 0.014 |
| Methylene blue . . . . . . . | 0–20 | 5.0 | **0.024** |
| | 20–40 | 8.0 | 0.039 |
| | 40–60 | 2.5 | 0.057 |

*Caffein.* — This was used hypodermically in two experiments (10 to 20 mg. per kg.) and intravenously, dissolved in $\frac{m}{7}$ $Na_2SO_4$, 25 c.c. per kg. of 0.04 per cent in two others. Both vagi were cut.[1] When used hypodermically it caused an insignificant rise if the chlorine was already low.

| Experiment XIX. | | | |
|---|---|---|---|
| | Time. | Diuresis. | Per cent. |
| Glucose . . . . . . . . . | .... | 1.75 | 0.103 |
| Caffein intravenously . . . | 0–20 | 24.5 | **0.045** |
| | 20–40 | 13.5 | 0.024 |
| | 40–60 | 5.5 | 0.022 |

[1] Anten, H.: Archives internationales de Pharmacodynamie, 1901, viii, p. 455.

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| EXPERIMENT XIII. | | | |
|---|---|---|---|
| | Time. | Diuresis. | Per cent. |
| Urea . . . . . . . . . . | .... | 2.0 | 0.040 |
| Caffein hypodermically . . | 0–20 | 6.0 | **0.062** |
| | 20–40 | 3.0 | 0.062 |
| | 40–60 | 1.5 | |

*Phlorhizin.*—This was given intravenously, 25 c.c. per kg. of 0.4 per cent solution, dissolved in one experiment in water, in another in $\frac{m}{7}$ $Na_2SO_4$. Example:—

| EXPERIMENT XIV. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | NaCl. | NaI. |
| $Na_2SO_4$, NaI . . . . . . | .... | 19.5 | per cent. 0.133 | per cent. 0.212 |
| Watery phlorhizin . . . | 0–20 | 21.0 | **0.060** | **0.064** |
| | 20–40 | 13.5 | 0.096 | 0.094 |

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6. **Effect of nephritic agents.** — Arsenate of soda, mercuric chloride, (one experiment each), and cantharidin (three experiments) did not affect the chloride retention. Examples:—

*Arsenate of sodium* in 1 per cent solution hypodermically.

| Experiment XIX. | | | | |
|---|---|---|---|---|
| | Time. | Diuresis. | NaCl. | Proteid. |
| | | | per cent. | |
| Glucose, caffein, $Na_2SO_4$ . . . . . | .... | 5.5 | 0.022 | None. |
| Arsenate, 10 mg. per kg. . . . . . | 0–20 | 0.5 | | |
| 25 c.c. per kg. $Na_2SO_4$ . . . . . . | 0–20 | 13.0 | 0.024 | Trace. |
| Arsenate, 20 mg. per kg. . . . . . | 0–20 | 15.0 | 0.030 | Less. |
| | 20–40 | 20.5 | 0.017 | Trace. |
| Repeated last dose . . . . . . . . | 0–20 | 10.5 | 0.013 | Considerable. |
| Repeated last dose . . . . . . . . | 0–20 | 8.0 | 0.018 | " |
| | 20–25 | 2.0 } | 0.018 | " |
| | 25–30 | 2.0 } | 0.018 | " |
| 25 c.c. per kg. $Na_2SO_4$ . . . . . . | 0–10 | 30.0 } | 0.018 | " |
| | 10–20 | 17.0 | 0.014 | " |
| Arsenate, 20 mg. per kg. intravenously | 0–20 | 8.5 | .... | " |

The urine stops 10 minutes later, but the animal remains alive for an hour longer.

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*Mercuric chloride.* — The dog has received daily hypodermic injections of 5 c.c. of 0.1 per cent $HgCl_2$, for the five days preceding the operation. Weight at operation, 8.9 kg.

| | Time. | Diuresis. | NaCl. | Proteid. |
|---|---|---|---|---|
| **Experiment XXIV.** | | | | |
| Bladder urine . . . . . | .... | .... | per cent. 0.050 | Trace. |
| Secreted while on table . . | .... | 1.6 | 0.156 | Trace. |
| 35 c.c. per kg. of $Na_2SO_4$ . | 0–10 | 34.0 | 0.018 | Very faint. |
| | 10–20 | 31.0 | 0.014 | Very faint. |
| **Cantharidin** [1] (10 mg. per kg.) | 0–10 | 24.0 | 0.018 | Very faint. |
| | 10–30 | 11.0 | 0.027 | Slightly more. |
| **Experiment XVIII.** | | | | |
| $N_2HPO_4$, caffein, $Na_2SO_4$ . | .... | 24.0 | 0.037 | None. |
| Cantharidin, 5 mg. per kg. . | 0–20 | 19.5 | 0.018 | Faintest trace. |
| Cantharidin, 10 mg. per kg. . | 0–20 | 7.5 | 0.016 | Appreciably more. |
| Died. | | | | |

[1] 1 per cent solution in acetic ether, given hypodermically.

*Experiment XXI.* — 5 mg. per kg. of cantharidin was injected on the day preceding the operation. The bladder urine contains considerable proteid, but no sugar. Very little urine secretion follows the injection of saline and other diuretics, but the per cent of chlorine is reduced as usual. The bladder urine contains 0.354 per cent NaCl; after injection of salines, the urine contains at first 0.108, later 0.090 per cent.

Kidneys suffering from **aloin nephritis** (produced by the hypodermic injection of 5 c.c. of 5 per cent aloin, daily, for three days) can also secrete a urine with a chlorine per cent lower than that of serum (0.27 per cent). The animal died before injections could be made.

**7. Other factors.** — Some other factors, which might be supposed to have an influence on the chloride excretion were observed incidentally.

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*Diuresis.* — The extent of the diuresis did not in any case materially modify the per cent of chlorine in the urine. Examples : —

| Experiment VII. | | Experiment XI. | | Experiment XVI. | |
|---|---|---|---|---|---|
| Diuresis. | NaCl. | Diuresis. | NaCl. | Diuresis. | NaCl. |
| | per cent. | | per cent. | | per cent. |
| 14.0 | 0.025 | 30.0 | 0.019 | 68.0 | 0.013 |
| 3.0 | 0.025 | 11.5 | 0.020 | 2.5 | 0.014 |

The per cent of chlorine is just as low with a small as with a large urine flow Examples : —

| Experiment X. | | Experiment XIV. | |
|---|---|---|---|
| Max. Diuresis. | NaCl. | Max. diuresis. | NaCl. |
| c.c. | per cent. | c.c. | per cent. |
| 7.5 | 0.021 | 33.0 | 0.027 |

*Time.* — The low per cent of chlorine in the urine outlasts the diuresis very much. In seventeen experiments it remained uniformly low for the hour, or longer, during which it was observed. In seven other experiments it shows a slight rise with time. This was so small (never exceeding 0.1 per cent) that it seems superfluous to risk any explanations. It may be due to the return of the composition of the blood toward the normal. Example : —

| Experiment I. | |
|---|---|
| Time after injection. | NaCl. |
| | per cent. |
| 0–17 min. | 0.035 |
| 17–58 " | 0.020 |
| 58–148 " | 0.060 |

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*Laking.* — This was observed in five experiments, as the result of the injection of watery solutions of urea, phlorhizin, or alcohol. It did not affect the chlorine in any of these experiments. Examples: —

| Experiment VII. | | | |
|---|---|---|---|
| | | NaCl. | NaI. |
| $Na_2SO_4$, glucose . . | No hæmoglobin . . | per cent.<br>0.030 | |
| Watery urea . . . | Hæmoglobin . . . | 0.025 | |
| **Experiment XIV.** | | | |
| $Na_2SO_4$, NaI . . . | No hæmoglobin . . | 0.133 | 0.212 |
| Watery phlorhizin . | Hæmoglobin . . . | 0.060 | 0.064 |
| **Experiment XXVII.** | | | |
| | | NaCl. | Time. |
| Original urine . . . | No hæmoglobin . . | per cent.<br>0.300 | min. |
| Watery alcohol . . | Hæmoglobin . . . | 0.103 | 0–20 |
| | | 0.043 | 20–40 |
| $Na_2SO_4$ . . . . . | Much less hæmoglobin | 0.092 | 0–10 |
| | " " | 0.019 | 30–50 |
| Ferrocyanide . . . | No hæmoglobin . . | 0.025 | |

## IV. Discussion of the Effects of the Various Factors on the Chlorides of the Urine, and the Light which they throw on the Mechanism of Chlorine Retention.

These factors may be divided into *four classes*: —

*Class I. Those which diminish the per cent of chlorides* (to 0.008, 0.060 per cent), *but which through increased diuresis cause a greater absolute amount of chlorine to be excreted in the urine.* This class comprises: Solutions of acetate, ferrocyanide, phosphate, and sulphate of sodium, urea, and glucose; as also the drugs of class III, if these are administered in solutions of the above salts.

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*Class II. Those which diminish the per cent of chlorine to the same degree, but which do not increase the urine secretion, and which therefore lower also the absolute amount of chlorine excreted by the urine.*

This class comprises water, and we could add, deficient chlorine income.

*Class III. Those which do not affect the per cent of chlorine materially; namely:*

(*a*) The diuretics which do not dilute the blood: caffein, phlorhizin, juniper oil.

(*b*) Nephritic agents: arsenic, cantharidin, mercury, ether, aloin.

(*c*) The degree of diuresis, laking of the blood, and (within the limit of the experiments) the time, and the molecular concentration and the quantity of fluid injected.

*Class IV. Those which greatly increase the per cent, and the absolute amount of chlorine in urines originally poor in this ion.* This class comprises: Nitrate, sulphocyanide, chloride, iodide (and probably bromide) of sodium.

The results are so constant, both qualitatively and quantitatively, that the mechanism by which they are produced must be comparatively simple. They agree entirely with those obtained through different methods by Loewi[1] with sodium nitrate, phlorhizin, and water, on dogs.

Very different results are obtained on the rabbit. Cushny[2] finds that in this animal the injection of $Na_2SO_4$ often increases the per cent of chlorine in the urine. Pototzky[3] finds that this is the case if the per cent of chlorine is originally low, whereas the per cent of chlorine is diminished if it was originally high. In either case the effect of the injection is to approximate the per cent of chlorine in the urine to that of the serum. The same results are seen in the rabbit, with caffein,[4] diuretin,[5] sugar,[6] urea,[7] phlorhizin,[8] sodium phosphate,[9] and nephritic agents.[10]

[1] Loewi, O.: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 410.

[2] Cushny, A. R.: Journal of physiology, 1902, xxvii, p. 429.

[3] Pototzky: Archiv für die gesammte Physiologie, 1902, xci, p. 565.

[4] Katsuyama, K.: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 235.

[5] Katsuyama, K.: *Loc. cit.*; Pototzky: *Loc. cit.*

[6] Pototzky: *Loc. cit.*

[7] Kasuyama, K.: *Loc. cit.*; Pototzky: *Loc. cit.*

[8] Ruschhaupt: Archiv für die gesammte Physiologie, 1902, xci, p. 565; Cushny, A. R.: *Loc. cit.*

[9] Loewi, O.: *Loc. cit.*

[10] Ruschhaupt: *Loc. cit.*

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Water and salt hunger being the only agents which uniformly lower the per cent of chlorine in rabbits' urine.

The difference between the results of Cushny and those obtained by Magnus and by myself is therefore explained, as I supposed[1] (p. 161) by the different animals which were employed. *The mechanism of chloride excretion reacts differently in rabbits and dogs.* I shall recur to this difference later (page 451) and confine myself at present to the experiments with dogs.

**1. What is the essential factor in the chlorine retention?** — If we confine our attention for the present to the first class of diuretics which diminish the per cent of chlorine in the urine, it will be seen that they possess the following factors in common: A dilution of the blood serum, and consequently a diminished per cent of chlorine in the serum; the presence of a foreign substance in the serum and in the urine; and an increased diuresis.

A comparison of Class I with Class II permits the elimination of all but one of these factors as unessential.

The increased diuresis is not essential, for it does not exist in Class II, and even in Class I the per cent of chlorine is quite independent of the diuresis. The presence of a foreign substance is not essential, for none is present in Class II; nor is the per cent of chlorine in the urine any lower if a mixture of chlorine and $SO_4$ is injected, than if a pure chlorine solution is administered. The dilution of the serum is not in itself essential, for it does not exist in the chlorine retention produced by salt-starvation.

*The essential factor in the production of the low per cent of chlorine in the urine is therefore the lowered per cent of this ion in the serum.*

**2. The per cent of chlorine in the urine is not determined by the total per cent of chlorine in the serum.** — The conclusion stated in the preceding paragraph is not supported by direct determinations of the chlorine in the serum. On the contrary, it is found that the urine remains poor in chlorine when the per cent of this ion in the serum has returned to normal; and that in some conditions (*i. e.* if $NO_3$ is introduced) the chlorine of the urine may be high when that of the serum has been lowered. I quote from some previous experiments.[2]

[1] SOLLMANN, T.: This journal, 1902, viii, p. 155.

[2] SOLLMANN, T.: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvi, p. 1.

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| Experiment VII (former series). | | |
|---|---|---|
| | NaCl in serum. | NaCl in urine. |
| | per cent. | per cent. |
| Before injection . . . . . . . . . | 0.57 | 0.105 |
| 50 min. after injection of $SO_4$ . . . | 0.45 | 0.040 |
| 3½ hours after injection of $SO_4$ . . . | 0.54 | $<0.030$ |
| **Experiment VI (former series).** | | |
| Before injection . . . . . . . . . | 0.56 | 0.427 |
| 3 min. after injection of $SO_4$ . . . | 0.43 | $<0.025$ |
| 3½ hours after injection of $SO_4$ . . . | 0.56 | 0.025 |
| **Nitrate Experiment.** | | |
| 1 min. after injection of $NO_3$ . . . | 0.37 | 0.400 |
| 1 hour after injection of $NO_3$ . . . | 0.43 | 0.26 |

In view of this contradiction, it is necessary to have recourse to Forster's hypothesis that the greater part of the serum chlorides ordinarily exists in the form of combinations (probably with the proteids) which are not capable of excretion by the urine, and that only the free chlorides can be excreted. It is true, as I have pointed out [1] (page 166), that the existence of such combinations has not been directly demonstrated.[2] Until this is done, the considerations

[1] Sollmann, T.: This journal, 1902, viii, p. 155.

[2] Buffa (Archives internationales de Pharmacodynamie, 1900, vii, p. 425) argues that there is such a combination from the fact that if serum is precipitated by ammonium sulphate and the precipitate is redissolved in the original quantity of water, this solution has the same $\Delta$ as the original serum. This shows, he believes, that the ammonium sulphate displaces the chlorine from its compound with the serum proteid. It seems to me that the experimental disposition, as far as can be judged from his meagre description and data, is too crude to allow any such far-reaching conclusions. His result might be pure coincidence. However, even if it be accepted, it would not explain the chlorine retention, for according to him the ammonium sulphate liberates the chlorine and would therefore make it filtrable.

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here laid down are amongst the strongest arguments for assuming their existence. The conclusion stated in the preceding paragraph must therefore be modified: *The essential factor in the production of a low per cent of chlorine in the urine is the lessened amount of unbound chloride in the serum.*

**3. Is the urine poor in chlorine secreted poor in this ion, or is it secreted with the normal content of chlorine, but modified after its secretion, by absorption of chlorine or by the secretion of water?** — If the urine were diluted after its secretion, the per cent of chlorine should vary according to the diuresis; if the low chlorine were due to a reabsorption of chlorine, then increased diuresis, leaving less time for this reabsorption, should give a urine richer in chlorine; if the low per cent were due to a secretion of water, then an increased diuresis, in which the secretion of water is increased, should yield a urine poorer in chlorine.

Since the diuresis does not affect the per cent of chlorine, the urine must be secreted poor in this ion. The extremely rapid urine formation also speaks against secondary changes. We may conclude that: *The urines poor in chlorides have a low per cent of this salt when they are first formed; the low per cent, in other words, is not due to reabsorption or to secondary dilution.*

**4. What is the mechanism by which urine with a low per cent of chlorine is formed from sera containing a low per cent of free sodium chloride, but a normal or only slightly subnormal per cent of total sodium chloride?**

The independence of the chlorine in the urine from the per cent of total chlorine in the serum has been sufficiently shown, so that it is not necessary to discuss a theory that the kidneys are impermeable to a low per cent of chlorine, but permeable to a large per cent.

Accepting then that the chlorine of the urine is determined by the free chlorine of the serum, we have the following theories:

1. The excretion of chlorine occurs by filtration, the chlorine of the filtrate corresponding to the free sodium chloride of the serum.

2. The excretion of chlorine occurs by secretion, the presence of free sodium chloride stimulating the renal (glomerular?) cells to the secretion of this salt.

A number of facts speak strongly against a pure filtration theory: (*a*) No physical filter is known which will effect this separation[1] (page 166).

[1] SOLLMANN, T.: *Loc. cit.*

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(*b*) The separation of such a urine would demand a greater filtration pressure than exists. This would not hold in the present case of salt injections, for quite enough foreign salt is present to make the total concentration of the urine superior to that of the serum. However, in water diuresis, this is not the case[1] (page 172).

(*c*) My former experiments[2] on chlorine injection also speak against a simple filtration. In these it is seen that the per cent of chlorine in the urine is superior to that of the serum, at a time when the quantity of serum in the body has returned to normal, and when therefore a considerable amount of the chlorine of the serum must be bound.

For instance: In Experiment IV one and one-half hours after injection the quantity of serum has returned to normal, the per cent of sodium chloride in the serum is 0.703; in the urine is 0.836.

This could only be explained on the filtration theory by assuming that a considerable reabsorption of water has taken place. If this were so, then the per cent of chlorine in the urine should be inversely proportional to the diuresis, for the greater the diuresis, the less would be the chance for the reabsorption of water. As a fact, however, the per cent of chlorine in the urine is practically independent of the degree of diuresis.

If filtration does not suffice to explain the phenomena of chloride excretion, *we are forced to assume a vital mechanism.*

I attempted to demonstrate this by studying the effect of renal stimulants and irritants on the chlorine excretion, which, if the process were a vital one, might be supposed to have an influence upon it. The attempt was unsuccessful, for no such influence was perceptible. This, of course, does not prove that the process is physical.

It still remains to explain several phenomena.

**5. The saline diuretics increase the absolute amount of chlorine, while they diminish its per cent.** — The saline diuretics, whilst they lower the per cent of chlorine in the urine, never cause its complete disappearance, and indeed increase the absolute quantity of chlorine excreted.

In accordance with the above theory this can only be explained by assuming that the diuretics increase the total amount of free sodium

[1] SOLLMANN, T.: *Loc. cit.*

[2] SOLLMANN, T.: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvi, p. 1.

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chloride. Since the per cent in the urine is independent of the amount of diuresis and of the amount of chlorine which has been removed, it would seem that the liberation of sodium chloride took place constantly. In other words, when the per cent of free sodium chloride in the serum tends to fall below a certain minimum, a further amount of sodium chloride is liberated from its combination.

**6. The effect of sodium nitrate, iodide, and sulphocyanide.** — Loewi,[1] who was only acquainted with the effect of sodium nitrate, assumed that it caused the passage of chlorine from the tissues into the serum. This is disproven by analysis of serum, which contains only 0.4 per cent sodium chloride. That this action plays only a small, if any part, is also shown by the fact that nitrate brings no more chlorine out of corpuscles than does sulphate.

A sample of fresh defibrinated dog's blood is mixed with 1½ volumes of 10 per cent $Na_2SO_4$ crystals; another sample with 1 volume of the sulphate and ½ volume of 15 per cent $NaNO_3$. The samples are centrifugalized. The sulphate serum contains 0.200 per cent NaCl, the nitrate serum 0.196 per cent.

The action must be something different. The similarity of these anions is suggestive: they are all monovalent, and dissociate in equal degree. It would seem that they are able to displace the sodium chloride from its unfiltrable combination, which the other anions are unable to do. The fact that sodium iodide and sodium chloride are excreted in practically equimolecular proportions, and that sodium iodide is affected in the same way as sodium chloride by diuretics, favors this view.

**7. Phlorhizin and nephritic poisons.** — The fact that phlorhizin and nephritic agents, which increase the per cent of sugar and of proteid in the urine, do not affect the chlorine, shows that they do not increase the general permeability of the kidneys and that their action is specific.

**8. The difference in the behavior of rabbits' and dogs' kidneys as regards the excretion of chlorine.** — The differences, which have been pointed out on page 446, would admit of the following explanations: —

1. The sodium chloride does not exist in unfiltrable combinations in rabbits' serum. This explanation cannot be the true one, since

[1] Loewi, O.: Archiv für experimentelle Pathologie und Pharmakologie, 1902, xlviii, p. 410.

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water, or salt-starvation, diminishes the chlorine in the rabbits' urine just as in dogs' urine.

2. The unfiltrable chlorine compounds, either in the serum or tissues, are broken up by all diuretics. Against this speaks the fact that diuretics *lower* the per cent of chlorine in the urine, if it was previously abnormally high.

3. *Diuretics break down the resistance of the kidney to the excretion of combined sodium chloride.* This seems the most likely hypothesis, and if we consider the greater susceptibility of the rabbits' kidneys to nephritic agents, it is not at all unlikely.

It would be interesting to know to which class the human kidney belongs? This could easily be determined by administering diuretics (except nitrate) on a milk diet.

## V. Conclusions.

1. *Effect of sodium sulphate injection on chlorine, Δ, and nitrogen.* The increased diuresis, as usual, tends to lessen the concentration of the urinary constituents, whilst it increases their absolute quantity. The per cent of chlorine, and the Δ, are but little affected by the degree of diuresis. The nitrogen and chlorine vary generally in the same direction, but by no means in the same proportion, the factor $\frac{N}{NaCl}$ varying between 2.0 and 7.9. This factor is independent of the degree of diuresis.

2. *Effect of various factors upon the chlorine of the urine, in the dog.* These factors may be divided into four classes.

I. Diminishing the per cent (to about 0.020 per cent) but increasing the absolute amount: solutions of urea, glucose, alcohol, sodium acetate, ferrocyanide, phosphate, and sulphate.

II. Diminishing the per cent and the absolute amount: water, salt-starvation.

III. Without effect: nephritic agents, caffein, phlorhizin, laking, degree of diuresis, and (within the limits of the experiments), the quantity or concentration of the injected fluid.

IV. Increasing the per cent of chlorine if this has been low: solutions of sodium nitrate, iodide, and sulphocyanide.

3. *Mechanism of the chloride retention.* The essential factor is the lowered quantity of unbound sodium chloride in the serum (not the

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absolute amount of sodium chloride in the serum, nor the dilution of the serum, nor the presence of foreign salts, nor the diuresis).

The low per cent of chlorine is mainly due to the urine being secreted poor in this salt, and not to secondary dilution, nor to reabsorption of chlorine.

The uninjured renal cells secrete only free sodium chloride, not combined sodium chloride. This property is not affected in the dog by diuretics or by nephritic poisons, whereas in the rabbit these agents cause the excretion of combined sodium chloride.

The sodium chloride is displaced from its combination by the nitrate, iodide, and sulphocyanide ions, but not by acetate, ferrocyanide, phosphate, sulphate, urea, or glucose.

Increased permeability of the kidneys to glucose or proteid is not necessarily accompanied by increased permeability to chlorides.

In conclusion, I wish to thank Mr. C. A. Lenhart and Dr. R. A. Hatcher for much valuable aid in carrying out the experiments.

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# THE COMPARATIVE DIURETIC EFFECT OF SALINE SOLUTIONS.[1]

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[*From the Pharmacological Laboratory, Western Reserve Medical College, Cleveland, Ohio.*]

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## I. Non-secretion of Urine.

As is common in a series of diuretic experiments on dogs, a number of the animals secreted practically no urine. This occurred in seven out of twenty-four experiments (29 per cent) in which diuretic injections were made. The urine of six of these non-diuretic animals contained proteid; in only one animal which gave a poor diuresis (Experiment X) was the urine free from proteids. In two of the animals (XX and XXI) the nephritis was due to the previous injection of nephritics (bichromate and cantharidin); in one (VIII), to the inspiration of liquid ether; in three (XIII, XXIX, and XXX) it was not accounted for.

It seems from these results that the main cause of anuria is nephritis. It can be seen, however, that not every form of albuminuria causes anuria. No anuria exists in the acute stage, immediately following the injection of the nephritic agent (cantharidin, Experiments XVIII and XXIV; alcohol, XVII; arsenate sodium,

[1] The experiments described in the preceding paper offered an opportunity for studying the rate of diuresis produced by these salts. The investigation was also extended to the phenomena of urine filtration which may be observed in the excised kidney.



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XIX; ether, XXV); and in subacute mercurial nephritis (Experiment XXIV).[1]

*The resistance of non-secreting kidneys to diuretic agents is* practically absolute. As examples I may quote Experiments XX and XXI. The animals had received: XX, 5 c.c. of 5 per cent $K_2Cr_2O_7$; XXI, 1 c.c. per kg. of 0.5 per cent cantharidin, hypodermically, a day before the operation.

Neither animal secreted any urine during an hour under anesthesia. They then received each the following injections, per kg., in the course of three hours: 140 c.c. $Na_2SO_4$ solution; 30 c.c. $Na_2HPO_4$ solution (= 170 c.c. of total fluid); 0.032 gm. citrated caffein; 0.4 gm. chloral; 0.016 gm. diuretin; 0.18 gm. urea. As the result of these, dog XX secreted in three hours 10 c.c. of urine (= 1.6 c.c. per kg.) and had a serous diarrhœa; XXI secreted in two and one-half hours 5.5 c.c. of urine (= 1 c.c. per kg.).

In one experiment (X) *section of the vagi relieved the anuria somewhat;* in VIII this was not effective; in the other five the vagi were divided before the injections were made.

*Experiment X.* —

11.25–12.20. No urine.
12.22. Injection of 35 per c.c. kg. $Na_2SO_4$; no urine to 12.41.
12.41. Injection of 35 per c.c. kg. $Na_2SO_4$; no urine to 1.10.
1.10. Divided both vagi.
1.17. *First drops of urine.*
1.37. 9.5 c.c. of urine in 20 min.
1.57. 15 c.c. of urine in 20 min. Injected 25 c.c. per kg. $Na_2HPO_4$.
2.17. 13 c.c. of urine in 20 min.
2.37. 6 c.c. of urine in 20 min.
2.57. 2 c.c. Injected 25 c.c. per kg. $NaC_2H_3O_2$.
3.37. No urine.

It was also noted, in the only experiment in which it was tried, that *hyperisotonic injections relieve the anuria somewhat.*

[1] Hellin and Spiro (Archiv für experimentelle Pathologie und Pharmakologie, 1897, xxxviii, p. 368) found that arsenate of potassium, and cantharidin, entirely suppressed the caffein and the phlorhizin diuresis in rabbits, whereas bichromate had no effect. This is another illustration of the difference between the dog's and rabbit's kidneys.

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*Experiment XXX* (only one kidney used). —

Before injections; no urine in 40 min.

Injection of 40 c.c. per kg. of 4.2 per cent $Na_2SO_4$ crystals per kg. in 30 min.; average urine in 10 min., 0.67 c.c.

Injection of 35 c.c. per kg. of 4.2 per cent $Na_2SO_4$ crystals per kg. in 26 min.; average urine in 10 min., 1.0.

Injection of 10 c.c. per kg. of 32.0 per cent $Na_2SO_4$ crystals per kg. in 50 to 65 min.; average urine in 10 min., 6.0.

## II. Influence of Glycosuria on the Initial Diuresis.

It was noticed that almost all good diuretic animals had a pronounced glycosuria, and vice versa. The sodium chloride per cent of the original urine, on the other hand, seemed to have no effect on the diuresis.

The influence of the glycosuria is well seen from the following epitome of the urines which were secreted before any injections were made:

*No sugar:* 9 cases; diuretic factor[1] from 0 to 1.9, mean 0.7.
*Small amount of sugar:* 3 cases; diuretic factor from 0.7 to 2.8, mean 1.5.
*Considerable sugar:* 11 cases; diuretic factor from 0 to 6.6, mean 2.0.

## III. The Time-relations of the Saline Diuresis.

The diuresis sets in very quickly after the injection of the saline solution, reaches its maximum usually in the first ten minutes, is maintained for about thirty minutes, and then declines quite rapidly. The amount secreted in ten minutes is often as much as 30 to 35 c.c. The onset of the diuresis occurs frequently during the injection. This is seen very strikingly in Experiment XIV. No urine had been secreted in the twenty minutes preceding the injection, but within a minute after starting the injection there appeared also the first drops of urine. In a few instances there was some delay, as in Experiment XVI, in which methylene blue in sodium sulphate was injected; the diuresis and blue appeared in ten minutes.

Repeated injections, after an interval of an hour, again restore the diuresis, and the same phenomena are observed as after the first injection. The diuresis is usually about the same for each injection, if the same solution is used; sometimes it is rather greater, and some-

[1] Cubic centimetres of urine secreted in one hour, divided by the weight of the animal in kilograms.

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times rather less in degree (Experiment XV), or less lasting (Experiment XVI). This is presumably due to the lowered circulation from the prolonged anesthesia.

## IV. Comparative Diuretic Effect of Various Diuretics.

The uniform technic employed in these experiments offers an excellent opportunity of comparing the diuretic effects. For this purpose I chose as *diuretic factor* of the maximum rate of diuresis the maximum number of cubic centimetres of urine secreted in forty consecutive minutes, divided by the weight of the animal in kilograms.

This factor disregards the rate of urine secretion before injection, for this has no appreciable effect upon the quantity of urine secreted in response to the diuretics. The period of observation is sufficiently long to eliminate accidental variations. The period of maximum diuresis is chosen rather than a definite period after the injection, to take account of the variations in the onset of the diuresis. As will be seen, this factor is really very constant, with different animals, if the same solution is used; it is much more uniform than any other factor which I have tried to apply, and it varies in a constant manner with different solutions. The factor is only computed to 0.5. All animals, the diuretic factor of which remained less than 5 after saline injections, are excluded as abnormal.

*Variations in the quantity of the injected solution (between* 25 *and* 75 *c.c. per kg.) have little effect upon the rate of diuresis :*

75 c.c. per kg. of $Na_2SO_4$ solution: 3 experiments; diuretic factor: extremes, 6 to 14.2; mean, 13.

25 and 35 c.c. per kg. of $Na_2SO_4$ solution: 9 experiments; diuretic factor: extremes, 10 to 20; mean, 13.

The same holds of other salts, or of successive injections; *i. e.* if several injections are given an hour apart, as was done in my experiments, the diuretic factor is practically the same for each injection.[1] This fact is very important, for it permits the comparison of all the injections, no matter in what order they were given. In the following table, the means of the results obtained on different animals are compared.

[1] It may be well to repeat that this diuretic factor relates only to the maximal rate of diuresis. It is quite probable that the diuresis is more prolonged and therefore absolutely greater when larger quantities of solution are injected. My experiments do not bear on this question.

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MAXIMAL RATE OF DIURESIS WITH THE VARIOUS DIURETICS.

25 to 35 c.c. per kg. of the solution, injected into the femoral vein in three minutes. The braces join those solutions which have practically the same diuretic factor.

| Solution. | Diuretic factor. | | No. of exp. |
|---|---|---|---|
| | Mean. | Extremes. | |
| $NaCl$ 0.5% | 1 | 0.5 to 2.5 | 3 |
| Alcohol 3% in water | 1¾ | 1.5 " 2.0 | 2 |
| $NaC_2H_3O_2$ $\frac{m}{7}$ | 4 | 1.5 " 6.5 | 2 |
| $Na_2SO_4$ $\frac{m}{14}$ with $NaCl$ 0.5% | 6 | 1.0 " 7.5 | 3 |
| $NaNO_3$ $\frac{m}{7}$[1] | 6 | .... | 1 |
| Glucose $\frac{m}{7}$ | 6½ | 6.0 to 6.5 | 2 |
| $NaSCN$ $\frac{m}{7}$ | 7½ | 6.0 " 8.5 | 3 |
| $NaI$ $\frac{m}{7}$ | 10 | 5.0 " 14.5 | 2 |
| Urea $\frac{m}{7}$ | 10 | 7.0 " 12.5 | 2 |
| $Na_2SO_4$ $\frac{m}{7}$ | 13 | 10.0 " 20.0 | 9 |
| $Na_2HPO_4$ $\frac{m}{7}$ | 13½ | 9.5 " 17.0 | 2 |
| $\frac{m}{7}$ $Na_2SO_4$ + alcohol 3% | about 15½ | .... | 1 |
| $\frac{m}{7}$ $Na_2SO_4$ + caffein 0.04% | " 17½ | 15.0 to 20.0 | 2 |
| $\frac{m}{7}$ $Na_2SO_4$ + juniper oil 0.4% | " 18 | .... | 1 |
| $\frac{m}{7}$ $Na_2SO_4$ + phlorhizin 0.4% | 18½ | .... | 1 |
| $Na_4Fe(CN)_6$ $\frac{m}{7}$ | 20½ | 16.0 to 24.5 | 2 |

[1] 75 c.c. per kg.

This table, deduced from the means, can be well *supported by the study of individual experiments* in which different salts were injected successively into the same animal:

*Water.* Less than $SO_4$, Experiment XXV.
*Alcohol in water.* Less than $SO_4$, Experiments XXV and XXVI.
*Acetate.* Less than $SO_4$ or $PO_4$, Experiments XI and XVII.
*Glucose.* Less than $SO_4$, Experiment XIX.
*SCN.* Less than $SO_4$ or $Fe(CN)_6$; more than alcohol in water, Experiments XXV and XXVI.

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*Iodide.* Less than $SO_4$, Experiments XIV and XV.

*Urea.* Less than $SO_4$, Experiment VII; more than glucose, Experiments VI and VII.

*$PO_4$.* Less than $SO_4$, Experiment XVIII; more than $C_2H_3O_2$ and $SO_4$, Experiment XI.

*$Fe(CN)_6$.* More than $SO_4$ and SCN, Experiment XXVI and XXVII.

*$SO_4$ and alcohol.* Greater than $C_2H_3O_2$, Experiment XVII.

*$SO_4$ and juniper.* Greater than $C_2H_3O_2$, Experiment XVII.

*Caffein in $SO_4$.* Greater than $SO_4$, Experiments XVI and XIX; than glucose, Experiment XIX.

*Phlorhizin.* Greater than $SO_4$, Experiment XVI.

## V. Causes of the Difference in Diuretic Effect.

The following table shows that the diuretic effect of the saline diuretics is generally proportional to the dissociation, *i. e.* is mainly a factor of the molecular concentration of the injected solution:

| Salt. | Mean diuretic factor. | In $\frac{m}{16}$ solution, 1 mol. dissociates into[1] |
|---|---|---|
| $NaC_2H_3O_2$ . . | 1 | 1.03 |
| $NaNO_3$ . . . | 6 | 1.8 |
| **Glucose** . . . | **6½** | 1.0 |
| NaSCN . . . | 7½ | 1.8 |
| NaI . . . . . | 10 | 1.8 |
| **Urea** . . . . | **10** | **1.0** |
| $Na_2SO_4$ . . . | 13 | 2.3 |
| $Na_2HPO_4$ . . | 13½ | 2.3 |
| $Na_4Fe(CN)_6$ . | 20½ | 2.6 |

[1] Compiled from Hamburger (Δ) and Ostwald (λ).

It is seen that a molecule dissociating into 2.6 molecules gives the highest diuresis; one dissociating into 1.8, less; 1.03, least. There are, however, breaks in the series: glucose, and especially urea, stand high; NaI stands above $NaNO_3$ or NaSCN.

The conclusion is therefore justified that the diuretic power of saline solutions is proportional, in the first place, to their freezing-

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point; and therefore in equimolecular solutions (in the sense of Hamburger) to their dissociation. Equiosmotic solutions, however, also vary. If this difference between equiosmotic solutions is expressed as "specific diuretic" power, then the specific diuretic power of urea and glucose surpasses the salts, urea surpasses glucose, iodide surpasses nitrate and sulphocyanide.

These conclusions appear in agreement with the results of Haake and Spiro,[1] although the differences in the methods makes comparison difficult.

## VI. How does the Molecular Concentration of the Injected Solution influence Diuresis?

The influence of the molecular concentration on diuresis which these experiments illustrate, was already shown by Münzer.[2] The explanation which has been given seems very simple. The hyperisotonic solution draws fluid from the tissues, so that the result is the same as if so much more fluid were injected.

This explanation is not sufficient in regard to the maximal rate of diuresis; for a substance dissociating into 2.6 molecules could increase the injected 25 c.c. of fluid to no more than 70 c.c.; and as has been shown (p. 457), such an increase in the amount of isotonic fluid would not alter the rate of diuresis. Some other explanation is necessary. This could be sought in a vital stimulation of the renal epithelium by the hyperisotonic solutions. However, it is not necessary to have recourse to such an obscure explanation, for the same phenomenon is seen on the perfusion of excised and dead kidneys with salt solutions.

*Method of the perfusion experiments.*—The kidneys were exposed by a large incision through the linea alba and another incision along the border of the ribs. A cannula was inserted into the ureter and another into the renal artery, toward the kidney, and connected at once with a bulb containing the warm perfusion fluid (saline solutions, with or without the addition of blood) placed at a height of 140 cm. above the animal. The perfusion was begun; a cannula was then placed in the renal vein, toward the kidney, and the kidney excised, the capsule being usually removed. An outflow of fluid from the ureter began at once, and con-

[1] Haake and Spiro: Beiträge zur chemischen Physiologie, 1902, ii, p. 149.

[2] Münzer, E.: Archiv für experimentelle Pathologie und Pharmakologie, 1898, xli, p. 74.

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tinued indefinitely. The rate of urine flow varied from $\frac{3}{4}$ to 22 c.c. in 10 minutes. That this fluid did not result from a rupture of the blood-vessels, is shown by: —

1. The low filtration pressure used.
2. If blood is circulated after the saline, the urinary fluid remains free from corpuscles.
3. The changes in the composition of the urinary fluid on changing the perfusing fluid are only complete after some 20 to 40 minutes.
4. The secretion varies with the vein outflow, the injection pressure remaining unchanged.
5. If blood diluted with $Na_2SO_4$ is circulated, the per cent of NaCl in the urine is generally less than in the serum.

The following typical experiment shows that *the rate of circulation and of urine formation varies with the osmotic pressure of the perfusing solution :*

| EXPERIMENT XXXVI. | | |
|---|---|---|
| Perfusing solution. | Flow from ureter in 10 minutes. | Flow from vein in 10 minutes. |
| | c.c | c.c |
| NaCl $\frac{3}{4}$% + $Na_2SO_4$ crystals 1 15% . | 6.0 | 95.0 |
| | 6.0 | 40.0 |
| NaCl 0.9% . . . . . . . . . . | 4.0 | 15.0 |
| | 1.7 | 11.3 |
| NaCl 1.95% . . . . . . . . . | 14.0 | 140.0 |
| | 18.0 | 190.0 |
| NaCl $\frac{1}{4}$% . . . . . . . . . . | 0.0 | 0.0 |
| $Na_2SO_4$ 8% . . . . . . . . . | 12.0 | 110.0 |
| | 9.0 | 90.0 |

The increased urine flow is simply a consequence of the increased circulation, for others of my experiments, which are reserved for a later paper, show that in the dead kidney, as in the living, the filtration of urine depends mainly upon the rate of perfusion, and but little on the filtration pressure. In the light of these experiments the superior *diuretic action of hyperisotonic solutions is explained very*

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*simply by their causing a shrinkage of the cells, and through this increasing the lumen of the blood-vessels, and hence the rapidity of the circulation.*

That we are dealing here with a purely physical phenomenon is shown by the fact that the changes of circulation are the same in kidneys which have been excised for three days.

| | Vein. | Ureter. |
|---|---|---|
| NaCl ¼% . . . . . | 26 | ½ |
| " 1.75% . . . . | 160 | 1 |
| | 122 | 1 |

*Strong alcohol* also increases the circulation.

| Experiment XXXVII. | | | | |
|---|---|---|---|---|
| | | Time. | Vein. | Ureter. |
| | NaCl 3.5% | 2.30 | 0 | 3 |
| | | 2.45 | 0 | 7 |
| | | 3.00 | 0 | 8 |
| 3.07 | Alcohol 80% | 3.13 | 26 | 2 |
| | | 3.15 | 26 | 2 |
| 3.16 | NaCl 2% | 3.25 | 10 | ⅓ |

*This result explains, on a physical basis, a number of phenomena which had hitherto to be regarded as proofs of "vital" secretory stimulations.*

(*a*) The superior diuretic action of hyperisotonic solutions, and the fact that intravenous injection of water has but little diuretic effect.

(*b*) Why the addition of a urinary constituent (harnfähige Substanz) increases diuresis. This has been noted by all observers who worked with sluggish kidneys. Munk[1], for instance, in his perfusion experiments, found very little secretion unless some such constituent was added. This he interprets as due to a stimulation; as he increased the concentration (NaCl was added to 2 per cent, etc.), the phenomenon is identical with that just described.

[1] Munk, I.: Archiv für pathologische Anatomie, cvii, p. 291.

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## VII. The Comparative Diuretic Effect of Diluted and Undiluted Blood.

*A blood diluted with saline solution circulates more rapidly, and consequently filters more urine, than an undiluted blood.* Examples: —

**Experiment XXXIII.**

| 1 part of defibrinated blood to | Flow from vein. | Flow from ureter. |
|---|---|---|
| ⅓ part isotonic saline solution | 27 | 0 |
| 1⅔ " " " " | 38 | few drops |
| | 60 | " |
| 4⅓ " " " " | 33[1] | " |
| 12 " " " " | 65 | ⅓ |
| 3 " " " " | 45 | 1 |
| | 35 | 1 |
| | 23 | ⅚ |
| | 12 | ½ |
| | 12 | $\frac{1}{10}$ |

[1] Really belongs to previous period.

**Experiment XXXVI.**
(The kidney has lain for two days.)

| | Flow from vein. | Flow from Ureter. |
|---|---|---|
| Pure saline . . . . | 96 | 18 |
| Blood 1, saline 6 . . | 26 | 3 |
| | 18 | 1 |
| | 0 | 0 |
| Pure saline . . . . | 12 | 2 |

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These two experiments, selected from a number of others,[1] illustrate a fact which is really self-evident, but which seems to have been neglected entirely by experimenters:

(*a*) Munk[2] found that ordinary defibrinated blood would not circulate through the excised kidneys, unless it had been diluted with saline solutions. He seeks the cause of this phenomenon in some clotting which, however, he could not demonstrate. The cause is simply that the undiluted blood is too viscid to circulate through the contracted vessels of the excised kidney.

(*b*) The diuretic effect of hydræmic plethora is partly, at least, explained by the dilution of the blood and the consequent more rapid renal circulation; conversely, the absence of diuresis on the injection of blood (Magnus[3]) is explained by the thickening of the blood and consequent slower renal circulation.

(*c*) It is not justifiable to estimate the rapidity of the circulation in the kidney by measuring the kidney volume, as is commonly done; for by dilution the renal circulation may be quickened without changing the calibre of the vessels; and by dehydration (hyperisotonic solutions) the calibre of the vessels may be altered without corresponding changes in the volume of the kidney.

## VIII. Conclusions.

1. The maximal rate of diuresis is fairly uniform for a given salt.

2. It is not markedly influenced by variations of 25 to 75 c.c. per kg. in the quantity of fluid injected.

3. In equimolecular solutions it varies generally with the number of dissociated ions, but urea has a greater diuretic effect than glucose: either is more active than the salts in equiosmotic solutions; the iodide is more diuretic than the nitrate or sulphocyanide.

4. The superior diuretic effect of hyperosmotic solutions cannot be satisfactorily explained by the greater hydræmia. The perfusion experiments show it to be due to increased circulation through the kidneys, produced by the dehydration of the renal tissues. Hypoisotonic solutions have the opposite effect.

[1] Some experiments are not very clear, because a solution containing a large amount of blood often obstructs the vessels so that the following solution does not circulate for a considerable time.

[2] Munk, I.: *Loc. cit.*

[3] Magnus, R.: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlv, p. 210.

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5. Hydræmic plethora increases diuresis, amongst other mechanisms, by diminishing the viscidity of the blood, and therefore increasing the circulation in the kidney.

6. The volume of the kidney is not a safe index of the circulation through this organ, even if the arterial pressure remain constant.

7. The urine filtration in the excised kidney depends much more upon the rapidity of the blood-flow than upon the arterial pressure.

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# THE RESPONSE OF THE FROG TO LIGHT.

By ELLEN TORELLE.

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## I. Introduction.

IN his study of light-response and color-sense of animals, Graber[1] states that frogs are negatively heliotropic. Loeb,[2] however, in his paper on the extension of heliotropic phenomena in the animal kingdom, finds them positively heliotropic.

[1] Graber: Grundlinien zur Erforschung des Helligkeits und Farbensinnes der Thiere, Prag, 1884.

[2] Loeb: Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen, 1890, p. 89.



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Loeb does not give a detailed account of his observations, but Graber gives tabulated results of experiments carried out between October 10 and 20. He used a large box about 2 cm. high and divided into two compartments, one of which was dark, the other illuminated with diffuse light (daylight). Three series of experiments of ten trials each were performed with Rana esculenta, forty frogs in each trial. The frogs were placed in the boundary between the light and the dark compartment, and each trial covered a period of fifteen minutes. The totals of the results are as follows:

| | I | II | III |
|---|---|---|---|
| Light . . . | 133 | 166 | 174 |
| Dark . . . | 267 | 234 | 226 |

indicating a reaction-proportion of 15:10.

Loeb finds that frogs move to the source of light, through whatever colored medium it be transmitted, a quantitative difference only being observable between the effects of lights of differing refrangibility. Graber had found, in three series of experiments of ten trials each, that 736 responded to the red, and 464 to the blue; the reaction-proportion being 6:10.

Loeb does not state whether or not he made observations with the intermediate colors, green and yellow. Graber, however, tested the response to green but not to yellow. As compared with red, the results in the two series of experiments were,

| I | | II | |
|---|---|---|---|
| Dark Red . . . | 450 | Bright green . . . | 350 |

the reaction-proportion being 10:13.

Compared with blue, he found the responses to be,

| I | | II | |
|---|---|---|---|
| Bright green . . . | 440 | Dark blue . . . | 280 |

the reaction-proportion being in this case 7:11. He compares the "attraction-strength"[1] of the colors, and finds them to be,

| Red. | Yellow. | Green. | Blue. |
|---|---|---|---|
| 1 | .. | 0.7 | 0.5 |

In order to carry out more detailed observations than have hitherto been made on the frog, and in order to determine conclusively, if

[1] GRABER attributed the response to differently colored lights as an exhibition of the *color-preference* of the animal.

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possible, its orientation to light, Dr. Morgan, to whom I am deeply indebted for kindly criticism, suggested that a series of experiments be performed to include responses of the frog to — (1) diffuse light, (2) direct light, (3) light reflected from below, (4) light transmitted from above, (5) light transmitted through a gelatine prism, (6) orientation with one eye covered, (7) orientation and reaction in high temperature and in low temperature, (8) response to monochromatic light.

The experiments were carried out in the Bryn Mawr biological laboratory from October to February, 1902–03. The material consisted of the two species, R. virescens virescens and R. clamata. The frogs were kept in an aquarium in the laboratory, where they seemed to remain in good condition.

In testing responses to light, two boxes were used as receptacles, the inner surfaces of which were painted a dull black, with the exception of the glass surface of one wall, through which the light was admitted. Each of the covers of the boxes contained a longitudinal slit, about an inch wide, permitting the movements of the frog to be observed without removal of the cover, a strip of black wadding which could be noiselessly lifted being laid over the slit.

At first, no time-limit was set for each trial, the frogs being kept in the box from ten to twenty minutes, or more, a varying number of frogs being used in each set of experiments. Later, all these experiments were repeated, a time-limit of ten minutes was set for each trial, and the same number of frogs was used in each set of experiments, except in cases where lack of conclusive response seemed to call for the trial of more individuals. Only one frog was used in each trial.

The results obtained fall into three divisions: Response to white light in the temperature of the laboratory; response to white light in increased and in lowered temperatures; response to monochromatic light.

## II. Response to White Light in the Temperature of the Laboratory.

**Diffuse light.** — The response to diffuse light was first observed; a tin box, nine inches long, five inches high, and twelve inches wide, prepared as above described, served as a receptacle. In the first set of experiments, six frogs were used. Each was placed at the

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rear, which was the darker end of the box, with the head turned away from the source of light. Though the time of response varied with each individual, as a rule, from one-fourth of one minute to one minute sufficed for turning and for moving the twelve inches to the opposite or light end of the box. While there was usually some movement from side to side of the box at this light end, the frog remained here during the rest of the time, which in this first set of experiments was twenty minutes or more. In all cases, whether moving or resting, the median plane of the body of the frog was parallel with the incoming ray.

In a second set of observations, made a month later, five frogs were used. Each trial lasted ten minutes, and the results were substantially as before.

These results seemed to indicate that the species of frog used were positively phototactic to diffuse light (daylight), and that in diffuse daylight the orientation of the frog was such that the median plane of the body was placed parallel to the incoming ray.

**Direct light.** — The apparatus used in the foregoing experiment was used also in determining the response to direct light. Five frogs were used in the first set of experiments, and five in the second. The sunlight fell into one end of the box only. In each case, the response was immediate and positive. The animals moved directly to the illuminated end of the box, where they remained a variable length of time, from two to four minutes, when they moved backward, just outside the circle of bright illumination, where they remained until taken away, the median plane of the body being parallel to the incoming ray. In most cases, when the sun-illuminated area was small, the head was not turned from the light during the retreat, which was accomplished by moving first one side of the body, then the other, sidewise and backward. In other cases the frogs turned at right angles to the light, hopped outside the area of intense illumination, and orientated themselves with their heads in the direction of the incoming ray.

Since the retreat into the area of less intense illumination might have been caused by the heat of the sun's rays, the experiments were repeated, heat being cut off by placing a glass vessel with parallel sides three and one-quarter inches apart and filled with water, close to the glass end of the box containing the frog to be tested. In each case, the result was practically the same as before. When the frog was placed in the rear of the box with its head directed from the

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source of light, it turned, moved into the sunlight close to the glass end, where it remained a short interval, and then retreated as before, remaining in a resting position in the area of lesser illumination until removed. When placed within the area of intense illumination, with the head directed toward the source of light, it left this region as before.

Tests were made out-of-doors, with the animals unconfined and free to move into a shadow from the sunshine or *vice versa*. Ten frogs were tried. Each was placed on a glass plate, covered with a bell-jar rendered impervious to light, and carried onto the lawn near the laboratory, where it was deposited about three yards from the shadow of the building, the bell-jar removed, and the frog left on the plate with its head turned away from the sun and from the shadow of the building.

The frog at first hopped forward, then stopped, turned in the direction of the sun, and hopped well into the shadow, where it remained quietly for ten minutes. It was then moved into the sunshine, in about its former position. Again it turned and hopped into the shadow. The results were very much the same in the case of each frog tried; there was a positive and decided movement from the sunshine into the shadow.

Since the sun's rays and the shadow of the building during these experiments were in exactly the same direction from the frog, it was impossible to decide whether the movement was due to a response to the direction of the ray or toward a shadow. Therefore, later in the day, when the shadow of the building became oblique to the direction of the sun's rays, the experiments were repeated, the frog being placed in such a position that if it moved into the shadow it must hop at right angles to the direction of the rays. In each case, the results were substantially these: First, the frog turned in the direction of the (sun) ray; second, it moved quickly into the shadow by a direct path. The experiments were repeated on different bright days, but the results were always the same as regards movement from the sun-illumined area into the shadow. In some instances the frog remained in the grass; in others, it moved close to the wall of the building.

The question now arose — Does the frog recognize the shadow as an area of less intense illumination, or would it move toward or onto a black surface as well if this were placed in the sunlight?

The side of a large wooden box was covered with black cloth, and

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the frog placed near the black perpendicular surface. It hopped close to this, remained but a couple of minutes, then moved to the wall of the gray-colored building, where it remained at rest in the angle formed by the wall and the ground. When placed near the uncovered box (pine) on the side in full sunlight, there was no movement toward it. When the box was raised on one edge and propped, so that the other edge was about four inches from the ground, the frog moved toward the shadow thus formed, crept well under the box, placed the body between its floor and the ground, where it remained with its head directed outward.

A black cloth was fastened close to the ground in the centre of a sun-illumined area, and a frog placed near it moved onto it, crept along the edge as if seeking cover, then hopped off. A second frog also hopped onto the cloth, but almost immediately moved off. Apparently a dark surface, brightly illuminated, does not produce the effect of a shadow or of diffuse light.

Tests were also made at mid-day on a level tract of ground about two acres in extent, which contained neither trees nor any object that could cast a shadow. Six frogs were tried. When freed, each moved indifferently toward any point of the compass, but usually kept on moving in the direction in which it began to move. In several trials no movement resulted; the frog crouched low between short bunches of grass, its head held close to the ground. When dark black or dark brown screens were placed in the middle of this area, and the frogs placed within five yards of them, the movement was toward and into the shadow of the screen, where they usually remained indefinitely.

**Diffuse light versus sunlight.** — A tin box eighteen inches long, three inches high, and three inches wide, painted a dull black inside and with the opposite ends, consisting of glass plates, placed so that the sun's rays were transmitted through one end and diffuse light through the other, was used as a receptacle. Five frogs were tried, each being given three trials, in each of which the first position in the receptacle was changed. That is, the frog was deposited first at the end at which diffuse light was transmitted, then in the middle of the box, then near the end at which sunlight was transmitted. In each case the frog turned toward and moved to the end at which the direct ray was transmitted, but did not remain within the circle of most intense illumination. In some cases it moved to the opposite end of the box; in others, without turning, it retreated into the area of less intense illumination.

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This experiment corroborates and reinforces the results obtained with diffuse light and direct light.

**Light reflected from below.** — In this set of experiments, a tin box, nine inches long, five inches high, and twelve inches wide, was used, all of whose surfaces were painted a dull black except the floor, which was made of window-glass. The box was supported so that the movements of the frog could be watched from below as well as from above. In the first set of experiments, five frogs were tried; in the second, fifteen. The results in both cases were the same, and differed with the amount of light (diffuse) admitted.

(*a*) When light was reflected from the whole area of the lower surface, the frog remained in normal resting position.

(*b*) When light was reflected from one-half of the lower surface, the frog hopped toward the light area.

(*c*) When the light was reflected from one-third of the surface, there was movement toward the light area, but the head was held at a greater angle to the horizontal.

In all the above trials it was found that the less the amount of light admitted, the greater the angle of the head to the horizontal plane of the floor of the box; so that, when light was reflected from the entire lower surface, a normal resting position was taken, about two-thirds of the ventral and posterior part of the body resting on the plate. When two-thirds of the lower surface of the box was covered (opaque to the light) only one-third of the ventral and posterior part of the body rested on the glass plate. In each case the frog moved from the darkened area onto or near the lighted area.

**Light transmitted from above.** — The box used in Experiment III was used here, the glass plate serving now as the upper side of the box. Eighteen frogs were tried; the average of results was about the same. In all cases the response to the direction of the incoming ray was immediate. The body was raised to an angle of about 45° to the horizontal. If a portion of the upper surface was covered, the frog moved to the uncovered side. Frequently, too, the frog jumped upward, toward the source of light.

Later, it was seen in experiments on five frogs, that the angle of inclination of the body varies as the distance of the frog from the upper illuminated surface. Each frog was placed in a tall glass jar which rested on a black cloth and was covered laterally by an opaque black cloth. If the entire lower and one-half of the lateral surfaces were covered, the angle of inclination of the frog's body was about 45°.

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If the entire jar was covered the body was raised so that the forelegs were as nearly as possible at right angles to the horizontal bottom of the jar. This made the inclination of the body 60° or over. Frequently the frog assumed an almost erect position, by means of placing the forefeet against the side of the jar. Some of these results can be demonstrated at any time by simply placing a frog in a tin pail and covering the pail with a wire gauze. The results are valuable here, together with those of the foregoing experiments, as showing that the frog is positively phototactic to light coming from any direction.

**Phototaxis in water.** — Is the frog positively phototactic in water? In order to answer this question a frog was placed successively in tubes of varying diameters, the smallest being one and three-eighths inches, one end closed with wire gauze, the tube placed at angles of inclination varying from 45° to a plane parallel with the floor of the receptacle, the end covered with gauze being held near the wall of the receptacle. Light was admitted from one end only, and the tubes were completely immersed in water. Five frogs were tried, each in three trials. All moved close to the illuminated end of the tube.

**Orientation.** — In the first five experiments, the floor of the receptacle was bare, being kept moist by occasional rinsings with cold water. It seemed desirable to ascertain if the movements and accuracy of orientation would be affected by the presence of a bank of sand or pebbles in the box, between the source of light and the frog. Upon six inches of the central longitudinal area and across the entire width of a box nine inches wide, twelve inches long, and five inches high, a bank of sand two and one-half inches high was made, the sides of which sloped gradually toward the darker and toward the illuminated ends of the box. Twelve frogs were tried. The movements of one frog will be followed as illustrative of the response of all.

The frog was placed in the rear compartment, with its head turned from the source of light. It immediately turned around, moved to the bank, where it paused, *crawled*, not hopped, up the bank to the top, then across the plane surface to the opposite edge, where it remained one and one-half minutes, then crawled down the bank and moved close to the glass at the light end of the box.

The same frog was again placed in the rear compartment after about one and one-half inches of water had been poured into it. It swam about at first, then crawled up the bank in the direction of the light, turned again toward the water, but soon moved to the lighted

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edge of the bank, where it remained four minutes, when it was removed and water poured into the lighted end of the box. Within one minute the frog had crawled over the bank and into the water at the lighted end, where it remained during the rest of the experiment, or nine minutes, moving from side to side of the box with its head against the glass end.

The responses of the other eleven frogs varied somewhat with the individual, but were in the main like the one above described.

**Light transmitted through a gelatine prism.** — A triangular prismatic plate, three inches in diameter at the base, was made by mixing lamp-black with dissolved gelatine and allowing it to become firm. This was then placed in front of the glass end of a box with the thick end of the prism to one side, so that light of differing intensities was admitted at the same time into the box. From time to time the position of the prism was reversed. In all cases the frog hopped to the side of the box at which the most light was transmitted, *i. e.* the thin part of the prism, with the median plane of the body in the direction of the incoming ray.

**The orientation of the frog with one eye covered.** — The left eye was first covered with black cambric of several thicknesses, cut and sewed together so as to fit smoothly over the left portion of the head, above the nostril and anterior to the tympanum. This cap-like garment was fastened to a cambric band passed around the body just posterior to the forelegs. The frog was placed in the box used in the former experiment, with its head directed from the source of light. It immediately turned, with its right eye directed toward the source of light, *i. e.* with its body oblique to the incoming ray. The angle of deviation from the direction of the incoming ray differed in different individuals. Five frogs were tried, but in no case was the orientation that observed when both eyes were uncovered.

Next, the right eye was covered in the same way that the left one had been, and now the frog orientated itself so that the left eye was directed toward the incoming ray. The frog was then removed from the box and allowed to jump freely on the floor; the movement was toward the left, and the frog alighted on the floor on one side, in an uncertain, floundering way.

In these experiments the responses were no doubt modified by the irritation caused by the covering, of which each frog tried to rid itself. That all the movements were due to this cause cannot be concluded, for in each set, when the right or the left eyes were covered

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the orientation was characteristically different, as if resulting from differing causes, and not merely similar movements caused by the irritation of the covering.

**Effect of prolonged light.** — Does exposure to light for a prolonged period alter the response to light-stimuli?

In order to answer this question two frogs were kept confined in glass-lined boxes two and one-half inches by one and one-half inches, the ends of which were covered with wire gauze. Since the frog turns in a very small space, cords were passed through the boxes from side to side and one-half inch from the top, forming a sort of fence, which allowed space for up and down movements of the head, but not for turning around. One frog was kept in the box from 11 A. M. to 4 P. M.; the other from 8.20 A. M. to 4.20 P. M. When freed and placed in the box with one lighted end, the response was the same as before, *i. e.*, the frogs were positively phototactic and also moved close to the lighted end of the box.

The foregoing experiments seem to indicate two different kinds of response to light. One kind, the response to the direction of the rays which affect orientation, is unquestionably phototaxis; the other I shall not venture to call photopathy in the present unsettled definition of the term.[1]

## III. Response to White Light in Increased and in Lowered Temperatures.

**The effect of increased temperature.** — The temperature of the aquarium in which the frogs were kept varied from 12° to 15° C. The temperature of the room in which the experiments were performed varied from about 18° to 20° C. In order to observe the effect of a rise in temperature on the character of the response to light, the box, before described, was placed within a large box, also having a glass end, and into which enough water could be poured to come well up the sides of the inner box. This water was heated by means of an Argand burner placed under the larger box. The temperature could be increased or kept constant as desired. A marked acceleration in time of response was noted in temperatures up to and including 25° C. The frog moved immediately and directly from the darker end of the box to the lighted end, where it remained close to

[1] Holmes, S. J.: This journal, 1901, iv, p. 211; Holt and Lee: This journal, 1901, ix, p. 460.

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the glass. Between 25° and 30° C. the frog became restless and moved about much. Above 30° C. movements toward the darker end were as frequent as those toward the lighter end, the response to light being overcome by the effect of heat.

**The effect of lowered temperature.** — In observing the effect of lowered temperature upon the response to light, the same apparatus was used as described for Experiment I b, *i. e.* a small tin box, twelve inches long, nine inches wide, and five inches high, containing a glass end, within a larger box sixteen inches long, twelve inches wide, and eight inches high, also furnished with a glass end. The bottom of the smaller box was covered with a layer of sand one-half inch thick, and the box was surrounded by ice placed in the larger box. The temperature of the water in the aquarium in which the frogs were kept was 15° C., the temperature of the box was 8° C. When a frog was placed in its rear end, head turned from the light, it moved to the light end once, remained there for one-half minute, but retreated, turned away from the light, and remained in the rear of the box, either moving about, its head down as if it were trying to get under something, or quietly crouching, with the head down during the other nine and one half minutes of the experiment. When returned to the aquarium, the above movements continued in the water, the frog remaining for several minutes on the floor of the aquarium. Five frogs were tried; three of these did not leave the rear end of the box at any time.

*Reaction in water to lowered temperatures.* — In order to test the reaction of the frog in water to lowered temperatures, a glass jar sixteen inches by eight inches in diameter was filled with water, and set in a box containing ice, so that the lower one-third of the surface was surrounded by ice; in other respects the jar was left entirely uncovered.

When the temperature of the water in the jar was 8° C. the frog was put into it. With swimming movements it went down almost immediately, head foremost, to the bottom of the jar. With legs outspread, almost at right angles to the longitudinal axis of the body, it moved about on the bottom of the jar, from time to time repeating the movements described as taking place in Experiment II b, but rarely coming to the surface.

From Experiments I, II, III b, one is led to conclude that an increase of temperature to 30° C. lessens the time of response to light, *i. e.* accelerates the rate; that below 8° C. the frog becomes negatively phototactic, whether it is in water or on a dry surface.

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**Stereotropism.** — If opportunity be given, does the frog burrow in sand in temperatures below 8° C., or are the movements observed stereotropic responses?

In order to answer this question, sand, to the depth of several inches, was placed in a tall glass jar, the jar being then filled with water. The sand was arranged so that its upper surface sloped from side to side. Twelve frogs were tried. When the temperature of the water in the jar became 10° C. the frogs went down and remained down, with the body flat and limbs outspread, but no attempt was made to burrow. The crouching movements, together with the passing of the head over the surface of the sand as if exercising a sense of touch, continued with a lowering of the temperature to 4° C., when they ceased. When a rock was lowered into the jar in such a way that a small space was formed between it and the wall of the jar, the frog crawled into this space and remained there. When a space was formed between the bottom of the jar and the rock, it crawled into that. This was tested several times, and was also observed when the temperature of the water in the aquarium in which the frogs were kept was lowered to 10° C. and below. When this was done, all the frogs responded, either by flattening their bodies against the stone floor, or by creeping under the rocks usually kept there. It therefore seems that the frog is stereotropic in temperatures between 10° C. and 4° C.

**Effect of darkness on upward and downward movements in water.** — The same jar used in the preceding experiment was used for this. The upper two-thirds of the jar including the open surface was covered with a cloth opaque to the light. With the temperature of the jar at 10° C. five frogs were tried, each being left in the jar ten minutes. They went immediately to the bottom, but rose to the top at intervals as before, and their movements seemed the same as when the jar was left uncovered. When the lower two-thirds of the jar was covered, no change was produced.

## IV. Response to Monochromatic Light.

The response to monochromatic light was studied in different ways. First, glass vessels with parallel sides three and one-half inches apart were used to hold solutions of pigment recommended by Davenport.[1]

[1] Davenport: Experimental morphology, p. 157.

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An alcoholic solution of fuchsin was used in testing the response to red; Lyon's blue, a concentrated solution of potassium chromate, and nickel nitrate were used for blue, yellow, and green respectively. The response to each was first separately observed.

A glass vessel containing a solution of fuchsin was placed close to the glass end of the tin box used in some of the previous experiments. This box was twelve inches long, nine inches wide, and five inches high, and the inner surfaces were painted a dull black; a slit in the cover, which was overlaid with several thicknesses of wadding, made frequent observations easy and caused little, if any, disturbance to the animal.

**Red.** — (*a*) When placed close to the red, the frog turned and hopped to the rear or opposite end of the box. This happened two out of seven times. Five times when so placed it turned away from the red, but remained in the front half of the box.

(*b*) When placed in the rear of the box it remained there six out of seven times. The seventh time it wandered about back and forth.

(*c*) When placed at or near the middle of the box, it was indifferent as to moving backward or forward. It usually remained about where it was placed.

**Yellow.** — The concentrated solution of potassium chromate was used in the same way that the fuchsin had been used. Five frogs were tried in ten trials. In each case, the frog moved to the source of the light, but soon retreated, remaining seated usually a short distance from it, indifferent as to orientation.

**Green.** — Four frogs were tried in twelve trials. There was much moving about, to and from the green, but in no case did the frog remain for any length of time close to the green light.

**Blue.** — Three frogs were tried in thirteen trials. The reaction was immediate and positive. Each frog hopped close to the glass, usually with the tip of its head against it, and frequently remained so until removed.

**Response to differently colored lights admitted at opposite ends of a receptacle.** — The question arose as to how the frog would respond were differently colored lights admitted into opposite ends of the receptacle. In order to answer this, a tin box eighteen inches long, three inches wide, and three inches high, whose inner surfaces were painted a dull black with the exception of the opposite ends, which consisted of glass, was used as a receptacle.

*Results.* — (*a*) The vessels before described were placed close to the

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glass ends. One was filled with a solution of fuchsin, the other with a solution of nickel nitrate. Five frogs were tried. Each moved from the red to the green or toward the green.

(*b*) When the green and the yellow lights were opposed, movement was from the yellow toward the green. The frogs usually remained a few inches from the green end of the box with heads turned toward the green light, but they were not always precisely orientated by the rays.

Five frogs were tried, each in two trials.

(*c*) Next yellow and red were used in the same way. Five frogs were tried. The movement was from red to yellow.

(*d*) When the blue light and the red light were at the opposite ends, the response was an immediate movement toward the blue. The difference between the response to the blue and the responses to the green and yellow is very marked. Blue not only effects an immediate response, but the frog remains close to the glass end where the blue solution is placed, frequently with its head against the glass and its median longitudinal axis parallel to the incoming ray.

TABLE I.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 8 min. | 2 min. | Head turned toward red and in red end of box. |
| 2 | 10 " | 0 " | Same as 1. |
| 3 | 10 " | 0 " | Same as 1. |
| 4 | 0 " | 10 " | Same as 1. |
| 5 | 0 " | 10 " | Same as 1. |
| 6 | 8 " | 2 " | Same as 1. |

**Unequal amounts of light transmitted through the red and through the blue media.** — Other tests were made with a greater amount of light transmitted through the red than through the blue medium. A vessel with parallel sides three and one half inches apart was used for the blue solution, one with its parallel sides one and one half inches apart being used for the red. In order to be able to make more accurate comparisons, it was thought best to note the time during which the frog in each trial remained with its head directed toward one or the

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other light, a ten-minute limit for each trial being taken. Since the responses to green and to yellow had seemed conclusive, and since previous observers differed as to the response to red and to blue, attention was confined to testing the response to these colors.

The results when red and blue were opposed are shown in Table I, showing a reaction-proportion of 4 : 2 in favor of the blue, even when more light was transmitted through the red medium.

**Response to a red and to a blue background.**— One-half of the inner surface of a tin box twelve inches long, nine inches wide, and five inches high was covered transversely with blue, one-half with red cheese-cloth. White light was admitted through the glass, at the end, which was covered with blue. The results are shown in Table II:

TABLE II.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog at the beginning of the experiment. |
|---|---|---|---|
| 1 | 7 min. | 3 min. | Head turned from light in the rear of red compartment. |
| 2 | 8 " | 2 " | Head turned from light in the middle of red compartment. |
| 3 | 8½ " | 1½ " | Head turned from light in the rear of red compartment. |
| 4 | 6 " | 4 " | Same as 3. |
| 5 | 5 " | 5 " | Same as 3. |

Then the strips were reversed, white light being admitted through the glass at the end, which was covered with red. The results are embodied in Table III.

TABLE III.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog at beginning of the experiment. |
|---|---|---|---|
| 1 | 10 min. | 0 min. | Head turned from light and in rear of blue compartment. |
| 2 | 10 " | 0 " | Same as 1. |
| 3 | 8 " | 2 " | Same as 1. |
| 4 | 9¾ " | ¼ " | Same as 1. |
| 5 | 9 " | 1 " | In the middle of the red area, head turned toward the light. |

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The box was lined with the strips laid lengthwise, the white light admitted equally on both; the position of the frog at the beginning of each trial, together with the results, being indicated in Table IV.

TABLE IV.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 9½ min. | ½ min. | In rear end of red compartment, head turned from light. |
| 2 | 9 " | 1 " | Same as 1. |
| 3 | 6 " | 4 " | Same as 1. |
| 4 | 9 " | 1 " | In rear of blue compartment, head turned from light. |
| 5 | 10 " | 0 " | Same as 4. |

Red and blue solutions were placed in front of the red and blue-lined sides of the box respectively, with results shown in Table V.

TABLE V.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog when experiment began. |
|---|---|---|---|
| 1 | 10 min. | 0 min. | In rear of red compartment, head turned toward the light. |
| 2 | 5 " | 5 " | In rear of red compartment, head turned from the light. |
| 3 | 7 " | 3 " | In middle of blue compartment, head at right angles to incoming light. |
| 4 | 10 " | 0 " | In rear of blue compartment, head toward light. |
| 5 | 10 " | 0 " | In rear of blue compartment, head turned from light. |

The results of the experiments with the colored cloths did not seem convincing, for the cloth offered an absorptive surface to the skin of the frog, and the dyes as well might have vitiating influences. So the external surfaces of three rectangular glass vessels were painted in the one case blue and red, transversely applied; in the other, the position of the colors was reversed; in the third, the paint was longitudinally applied.

The blue corresponded to the tube paint known as new blue; the red, to vermilion. White light was transmitted at the open end through an ordinary window-pane.

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(*a*) The vessel on which the paints were longitudinally applied was first used.

TABLE VI.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 10 min. | 0 min. | In rear of red compartment. |
| 2 | 8 " | 2 " | In rear of red compartment. |
| 3 | 3½ " | 6½ " | In rear of blue compartment. |
| 4 | 10 " | 0 " | In middle of blue compartment. |
| 5 | 10 " | 0 " | In middle of red compartment. |
| 6 | 10 " | 0 " | In rear of blue compartment. |
| 7 | 8 " | 2 " | In middle of red compartment. |
| 8 | 0 " | 10 " | In rear of red compartment. |
| 9 | 10 " | 0 " | In rear of blue compartment. |
| 10 | 9½ " | ½ " | In ant. end of red compartment. |
| 11 | 8 " | 2 " | In ant. end of red compartment. |
| 12 | 9 " | 1 " | In rear end of red compartment. |
| 13 | 10 " | 0 " | In rear end of blue compartment. |

The above table (Table VI) shows a response of eleven out of thirteen to blue. The response to red may be accounted for by fear, or by sluggishness.

(*b*) Next the vessels to which the colors had been transversely applied were used. The frog was first placed in the vessel in which the red was next to the white light. It was watched for ten minutes; then this same frog was put into the aquarium in which the blue was next to the white light and again watched ten minutes. The frog was always placed in the same relative positions in the two vessels and the positions differed for each experiment.

When red was next to the white light the responses were those indicated in Table VII:

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TABLE VII.

| No. of frog. | Time at blue end. | Time at red end. | Position of frog when experiment began. |
|---|---|---|---|
| 1 | 10 min. | 0 | In right-hand, rear corner of blue. (Moved to red boundary and stopped.) |
| 2 | 10 " | 0 | In anterior part of blue compartment. (Moved to red boundary and stopped.) Remained in blue compartment. |
| 3 | 8 " | 2 | In middle of red compartment. |
| 4 | 9½ " | ½ | In rear of middle part of blue compartment. |
| 5 | 10 " | 0 | In rear of left-hand corner of blue compartment. |
| 6 | 8 " | 2 | In middle of red compartment. |

When blue was next to the white light the frogs responded as shown in Table VIII:

TABLE VIII.

| No. of frog. | Time on blue. | Time on red. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 0 min. | 10 min. | In middle of red compartment. |
| 2 | 10 " | 0 " | In middle of blue compartment. |
| 3 | 7 " | 3 " | In middle of blue compartment. |
| 4 | 10 " | 0 " | In rear of red compartment. |
| 5 | 0 " | 10 " | In rear of red compartment. |
| 6 | 10 " | 0 " | In middle of blue compartment. |

A constancy in the response of the same frog, in vessels in which the colors are reversed, is here observed, except in frogs 1 and 5 of the last set. The first I can account for, as the glass plate at the open end fell during the experiment and frightened the frog into retreat; the case of the fifth I cannot account for except that its condition was rather sluggish.

A week later the same experiments were repeated with the following results:

When red is next to the white light the following results were obtained:

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TABLE IX.

| No. of frog. | Time on blue. | Time on red. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 10 min. | 0 min. | In rear of blue compartment. |
| 2 | $9\frac{1}{2}$ " | $\frac{1}{2}$ " | In rear of blue compartment. |
| 3 | $9\frac{1}{2}$ " | $\frac{1}{2}$ " | In middle of red compartment. |

When blue is next to the white light the results are those indicated in Table X:

TABLE X.

| No. of frog. | Time on blue. | Time on red. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 8 min. | 2 min. | In rear of red compartment. |
| 2 | 10 " | 0 " | In rear of red compartment. |
| 3 | 2 " | 8 " | In middle of blue compartment. |

The experiments were repeated, using other frogs, and changing the time of each trial from ten to twenty minutes. When red is next to the source of light the behavior of the frog is that indicated in Table XI.

TABLE XI.

| No. of frog. | Time on blue. | Time on red. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 20 mins. | 0 mins. | In middle-left of red compartment. |
| 2 | 20 " | 0 " | In middle-left of red compartment. |
| 3 | 2 " | 18 " | In middle-left of red compartment. |

When blue was next to the source of light the frogs responded as indicated in Table XII:

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TABLE XII.

| No. of frog. | Time on blue. | Time on red. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 16 min. | 4 min. | In middle of blue compartment. |
| 2 | 9 " | 11 " | In middle-left of blue compartment. |
| 3 | 12 " | 8 " | In middle of blue compartment. |

Calculating in minutes the response to a red or to a blue background, when white light is admitted, the response to the blue was three times greater than that to the red, the actual number of minutes on the blue being 479; on the red, 159.

**Response when the entire environment is one-half blue and one-half red.** — A glass plate, one-half of which was painted red, the other one-half blue, was placed before the opening of the vessel[1] so that the red of the plate was adjacent to the red of the vessel, and the blue of the plate adjacent to the blue of the aquarium. The trials were of twenty minutes' duration and gave the following results:

TABLE XIII.

| No. of frog. | Time on blue. | Time on red. | Position of frog when placed in the vessel. |
|---|---|---|---|
| 1 | 16 min. | 4 min. | In rear of red compartment. |
| 2 | 20 " | 0 " | In rear of blue compartment. |
| 3 | 17 " | 3 " | In middle of red compartment. |
| 4 | 20 " | 0 " | In middle of blue compartment. |
| 5 | 20 " | 0 " | In front end of blue compartment. |
| 6 | 19 " | 1 " | In front end of red compartment. |

In this set of experiments the reaction-proportion is as 14 : 1 in favor of the blue.

**Response when white light is admitted at opposite ends of a receptacle, one-half of the surface of which is painted red, the other half blue.** — A tin box eighteen inches long, three inches wide, and

[1] In which the strips of red and blue ran lengthwise.

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three inches high, and containing opposite glass ends (three inches by three inches), was laid off into equal compartments and its inner walls painted blue and red. In six trials the same frog was used, being put in the same relative position but upon a different color in consecutive experiments. In eleven trials the colors were reversed after each experiment, the frog being placed now on red, now on blue, and again on the boundary between the two. The results of the eleven trials are shown in the following table:

TABLE XIV.

| No. of frog. | Time on blue. | Time on red. | Position of frog at beginning of experiment. |
|---|---|---|---|
| 1 | 10 min. | 0 min. | In rear of red compartment. |
| 2 | 10 " | 0 " | In front end of blue compartment. |
| 3 | 10 " | 0 " | In middle of blue compartment. |
| 4 | 10 " | 0 " | In rear of blue compartment. |
| 5 | 9 " | 1 " | In front end of red compartment. |
| 6 | 9 " | 1 " | In middle of red compartment. |
| 7 | 8 " | 2 " | In rear of red compartment. |
| 8 | 7½ " | 2½ " | In front end of red compartment. |
| 9 | 6½ " | 3½ " | In middle of red compartment. |
| 10 | 6 " | 4 " | In rear of red compartment. |
| 11 | 9½ " | ½ " | In rear of blue compartment. |

The results in the former case show a greater length of time on the blue in the case of all except the second frog, which, as indicated in Table XV, remained in the red compartment for ten minutes when placed in the red compartment.

The results obtained in response to monochromatic light seem to illustrate Loeb's theory " that the more refrangible rays are extraordinarily more active than the less refrangible, which occasionally remain almost ineffective." [1] According to Abelsdorff,[2] red rays affect the pupils of the eyes of some animals like darkness.

[1] Loeb, J.: Der Heliotropismus der Thiere, p. 20.

[2] Abelsdorff, G.: Archiv für Physiologie, 1900, p. 561.

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Graber's result on the frog can be explained only on the ground of a confusion arising as a result of using so many frogs (forty) at the same time in one receptacle.

TABLE XV.

| No. of frog. | No. of trials. | Time on blue. | Time on red. | Position of frog at the beginning of the experiment. |
|---|---|---|---|---|
| 1 | 1 | 10 min. | 0 min. | At glass end of blue compartment. |
| | 2 | 10 " | 0 " | At glass end of blue compartment. |
| 2 | 1 | 9 " | 1 " | At glass end of red compartment. |
| | 2 | 0 " | 10 " | At glass end of red compartment. |
| 3 | 1 | 8 " | 2 " | At glass end of blue compartment. |
| | 2 | 9½ " | ½ " | At glass end of blue compartment. |

## Conclusions.

1. At the usual temperature of the laboratory, between 16° and 21° C., Rana virescens virescens and R. clamata are positively phototactic.

2. They respond to light coming from above and from below, as well as from the side.

3. They respond differently to different intensities of light. They move out of the sunlight into the shadow, even when by so doing the movement is away from, or at right angles to, the direction of the ray.

4. When one eye is covered, the body is placed with its median plane oblique to the ray.

5. When a bank of sand is interposed between the frogs and the light, they crawl over this and move to the source of light.

6. A rise in the temperature to 30° C. accelerates the rate of the positive response. A lowering of the temperature to 10° C. produces movements away from the light.

7. When placed in water the temperature of which is lowered to 10° C., the frogs swim downward: (*a*) In an uncovered glass vessel; (*b*) In a vessel, the upper two-thirds of which has been darkened; (*c*) In a vessel the lower two-thirds of which has been darkened.

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8. The frogs turn away from red light and move toward blue light. They move toward green and toward yellow light, but are not definitely orientated by either.

9. When red light is admitted at one end and green light at the other end of a receptacle, the frogs move from the red to or toward the green. When red and yellow lights are opposed in the same way, movement is from the red to the yellow. When red and blue are opposed, movement is immediately toward the blue.

10. When white light is admitted at one end of a receptacle, and the frogs are given a choice of a red or of a blue environment, they move, in most cases, into the blue, and remain in it longer than they do in the red.

11. When one-half of the entire receptacle is blue and the other half is red (no white light being admitted), movement is from the red to the blue.

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